NATIONAL INSTITUTES OF HEALTH

GUIDELINES FOR

RESEARCH INVOLVING RECOMBINANT DNA MOLECULES

APPENDIX D

SUPPLEMENTARY INFORMATION ON PHYSICAL CONTAINMENT

JUNE 1976

INDEX

## Supplementary Information on Physical Containment

### I. Biological Safety Cabinets

Biological Safety Cabinets suitable for confining operations involving recombinant DNA molecules are described below:

1. Class I - A ventilated cabinet for personnel protection only, with an unrecirculated inward flow of air away from the operator. The exhaust air from this cabinet may be filtered through a high-efficiency or high-efficiency particulate air (HEPA) filter before being discharged to the outside atmosphere. This cabinet is suitable for research work with the Center for Disease Control (CDC) classes of etiologic agents 1, 2 and 3 where no product protection is required. This cabinet may be used in three operational modes: (i) with an eight-inch high, full-width open front; (ii) with an installed front closure panel (having four, eight-inch diameter openings) without gloves; and (iii) with an installed front closure panel equipped with arm length rubber gloves. See Table I for ventilation requirements, agent use limitations, and minimum performance requirements.

2. Class II - A ventilated cabinet for personnel and product protection having an open front with inward air flow for personnel protection, and HEPA-filtered recirculated mass air flow for product protection. The cabinet exhaust air is filtered through a HEPA filter. Two models of this cabinet are available, Type 1 and Type 2.

   (i) Type 1 - The Type 1 recirculates approximately 70% of the air. The exhaust air from this cabinet may discharge into the laboratory or be diverted out of the laboratory. This cabinet is suitable for CDC classes of etiologic agents 1, 2, and 3. Vapors or gases which are hazardous from a toxic, radioactive, or flammability standpoint should not be used in this cabinet because of the high quantity of recirculated air.

   (ii) Type 2 - The Type 2 cabinet recirculates approximately 30% of the air. The exhaust air from this cabinet is normally ducted out of the laboratory through a HEPA filter and, occasionally, an activated charcoal filter depending on the operation. The cabinet may be used with gases or vapors that are hazardous from a

toxic, radioactive, or flammability standpoint. However, any consideration of use of such materials should be evaluated carefully from the standpoint of build-up to dangerous levels and problems of decontamination of the cabinet. See Table I for ventilation requirements, agent use limitations, and minimum performance requirements.

3. Class III - A closed front ventilated cabinet of gas-tight construction providing total protection for personnel and product from contaminants exterior to the cabinet. The cabinet is operated under a negative pressure of at least 0.5 inches water gauge. All supply air is HEPA-filtered. Exhaust air is HEPA-filtered or incinerated to protect the environment. This cabinet, fitted with arm length rubber gloves, provides the highest containment of these three classes of cabinets and is utilized for all activities involving high risk agents (i.e., CDC etiologic agents, class 4). See Table I for ventilation requirements, agent use limitations, and minimum performance requirements.

The integrity of any cabinet depends on initial and periodic evaluation to meet established performance tests. Table I outlines the minimum performance required to assure that the cabinets will provide protection of personnel and the environment.

TABLE I

BIOLOGICAL SAFETY CABINETS

SAFETY PERFORMANCE REQUIREMENTS AND SPECIFICATIONS
JUNE 1976

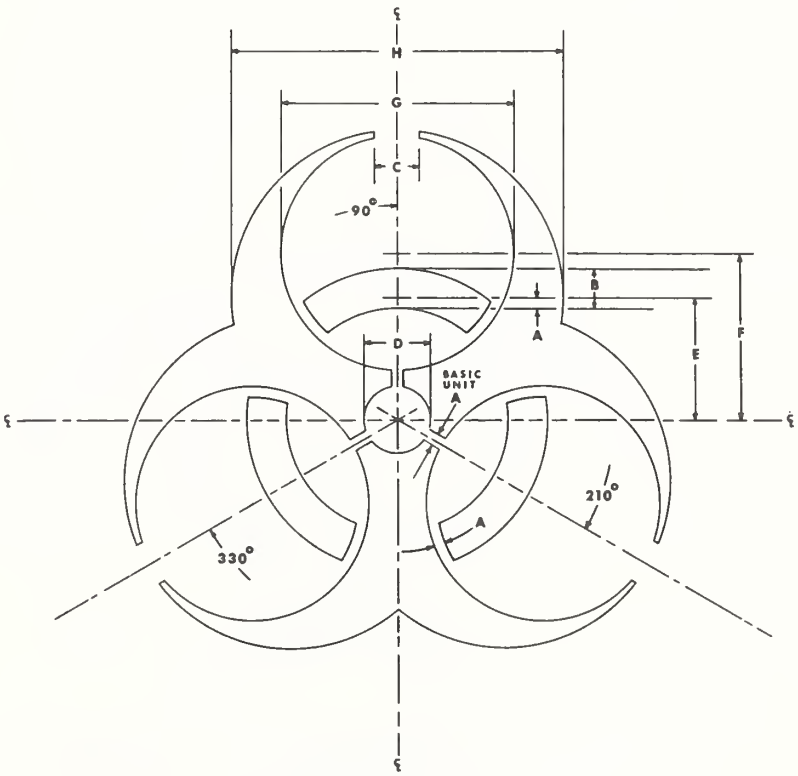
| CABINET | USE CLASSIFICATION | | PERFORMANCE REQUIREMENTS | | | | |
|---|---|---|---|---|---|---|---|
| | DNA[a] | CDC[b] | FACE VELOCITY (linear feet per minute) | EXHAUST AIR (CFM)[c] 4'hood | 6'hood | LEAK TIGHTNESS | EXHAUST FILTER EFFICIENCY |
| Class I | P1-P3 | 1-3 | 75 | 200 | 300 | Not applicable | 99.97% |
| Class II, Type 1 | P1-P3 | 1-3 | 75 | 260 | 400 | Gas tight; Leak rate < $1x10^{-5}$ cc/sec at 2"wg pressure | 99.97% |
| Class II, Type 2 | P1-P3 | 1-3 | 100 | 250 | 360 | Pressure tight; No air/soap bubble at 2"wg pressure | 99.97% |
| Class III | P4 | 4 | Not applicable | d | d | Gas tight; Leak rate < $1x10^{-6}$ cc/sec at 3"wg pressure | 99.97% |

a - For work with recombinant DNA molecules.
b - Center for Disease Control (US Public Health Service).
c - CFM=cubic feet per minute.
d - Based on one volume of air change each 3 minutes, in the absence of unusual heat or moisture that would require more air changes.

II. Universal Biohazard Warning Symbol (1)

The biological hazard warning symbol (biohazard symbol) specified herein shall be used to signify the actual or potential presence of a biohazard and to identify equipment, containers, rooms, materials, experimental animals or combinations thereof which contain or are contaminated with viable hazardous agents.

The biohazard symbol shall be designed and proportioned as illustrated here:




| DIMENSION | A | B | C | D | E | F | G | H |
|---|---|---|---|---|---|---|---|---|
| UNITS | 1 | 3½ | 4 | 6 | 11 | 15 | 21 | 30 |

The symbol shall be as prominent as practical, and of a size consistent with the size of the equipment or material to which it is affixed, provided the proportions shown above are maintained, and, in any case, that the symbol can be easily seen from as many directions as possible.

Except when circumstances do not permit, the symbol shall be oriented with one of the three open circles pointed up and the other two forming a base.

The symbol color shall be a fluorescent orange or orange-red color.* Background color is optional as long as there is sufficient contrast for the symbol to be clearly defined.

BIOHAZARD

Revised 9-9-66

*Day-Glo$^{R}$ Fire Orange of the Switzer Brothers, Inc. is cited as an example, not an endorsement.

The biohazard symbol shall be used or displayed only to signify the actual or potential presence of biological hazard.

Appropriate wording may be used in association with the symbol to indicate the nature or identity of the hazard, name of individual responsible for its control, precautionary information, etc., but never should this information be superimposed on the symbol. (See next page)

# ADMITTANCE TO AUTHORIZED PERSONNEL ONLY

**Hazard identity:** _____

**Responsible Investigator:** _____

**In case of emergency call:**

**Daytime phone** _____ **Home phone** _____

**Authorization for entrance must be obtained from the Responsible Investigator named above.**

## III. Laboratory Techniques For Biohazard Control

### A. Pipetting

1. No infectious or toxic materials should be pipetted by mouth (2,3,4).
2. No infectious mixtures should be prepared by bubbling expiratory air through a liquid with a pipette (2,3,4).
3. No infectious material should be blown out of pipettes (2,3,4).
4. Pipettes used for the pipetting of infectious or toxic materials should be plugged with cotton (2,3,4).
5. Contaminated pipettes should be placed horizontally in a pan containing enough suitable disinfectant to allow complete immersion (2,3,4). They should not be placed vertically in a cylinder.
6. The pan and pipettes should be autoclaved as a unit and replaced by a clean pan with fresh disinfectant (2,3,4).
7. Infectious material should not be mixed by alternate suction and expulsion through a pipette (2,3,4).
8. Mark-to-mark pipettes are preferable to other types, as they do not require expulsion of the last drop (5).
9. Discharge should be as close as possible to the fluid or agar level, or the contents should be allowed to run down the wall of the tube or bottle whenever possible--not dropped from a height (5).

10. A disinfectant-wetted towel over the immediate work surface is useful in some cases to minimize the splash from accidental droppage (9).

B. Syringes and Needles (9)

1. To lessen the chance of accidental injection, aerosol production or spills, avoid unnecessary use of the syringe and needle. For instance:

   (i) Use the needle for parenteral injections but use a blunt needle or a cannula on the syringe for oral or intranasal inoculations.

   (ii) Do not use a syringe and needle as a substitute for a pipette in making dilutions of dangerous fluids.

2. Use the syringe and needle in a Biological Safety Cabinet only and avoid quick and unnecessary movements of the hand holding the syringe.
3. Examine glass syringes for chips and cracks, and needles for barbs and plugs. Note: This should be done prior to sterilization before use.
4. Use needle-locking (Luer-Lok$^{R}$ type) syringes only, and be sure that the needle is locked securely into the barrel. A disposable syringe-needle unit (where the needle is an integral part of the unit) is preferred.

5. Wear surgical or other type rubber gloves for all manipulations with needles and syringes.
6. Fill the syringe carefully to minimize air bubbles and frothing of the inoculum.
7. Expel excess air, liquid and bubbles from a syringe vertically into a cotton pledget moistened with the proper disinfectant, or into a small bottle of sterile cotton.
8. Do not use the syringe to expel forcefully a stream of infectious fluid into an open vial or tube for the purpose of mixing. Mixing with a syringe is condoned only if the tip of the needle is held below the surface of the fluid in the tube.
9. If syringes are filled from test tubes, take care not to contaminate the hub of the needle, as this may result in transfer of infectious material to the fingers.
10. When removing a syringe and needle from a rubber-stoppered bottle, wrap the needle and stopper in a cotton pledget moistened with the proper disinfectant. If there is danger of the disinfectant contaminating sensitive experiments, a sterile dry pledget may be used and discarded immediately into disinfectant solution.
11. Inoculate animals with the hand "behind" the needle to avoid punctures.

12. Be sure the animal is properly restrained prior to the inoculation, and be on the alert for any unexpected movements of the animal.
13. Before and after injection of an animal, swab the site of injection with a disinfectant.
14. Discard syringes into a pan of disinfectant without removing the needle. The syringe first may be filled with disinfectant by immersing the needle and slowly withdrawing the plunger, and finally removing the plunger and placing it separately into the disinfectant. The filling action clears the needle and dilutes the contents of the syringe. Autoclave syringes and needles in the pan of disinfectant.
15. Use separate pans of disinfectant for disposable and non-disposable syringes and needles to eliminate a sorting problem in the service area.
16. Do not discard syringes and needles into pans containing pipettes or other glassware that must be sorted out from the syringes and needles.

C. Opening Culture Plates, Tubes, Bottles, and Ampoules

1. Plates, tubes and bottles of fungi may release spores in large numbers when opened. Such cultures should be manipulated in a Biological Safety Cabinet (6,15).

2. In the absence of definite accidents or obvious spillage, it is not certain that opening of plates, tubes and bottles of other microorganisms has caused laboratory infection. However, it is probable that among the highly infective agents, some infections have occurred by this means and are represented in the 80% for which no known act or accident is ascribable (3).
3. Water of syneresis in petri dish cultures is usually infected and forms a film between the rim and lid of the inverted plate. Aerosols are dispersed when this film is broken by opening the plate. Vented plastic petri dishes where the lid touches the rim at only three points are less likely to offer this hazard (8,19).
4. The risk may also be minimized by using properly dried plates, but even these (when incubated anaerobically) are likely to be wet after removal from an anaerobic jar. Filter papers fitted into the lids reduce, but do not prevent, dispersal. If plates are obviously wet they should be opened in the Biological Safety Cabinet (8).
5. Less obvious is the release of aerosols when screw-capped bottles or plugged tubes are opened. This happens when a film of infected liquid which may collect between the rim and the liner is broken during removal of the closure (8).

6. Dried, infected culture material may also collect at or near the rim or neck of culture tubes and may be be dispersed into the air when disturbed (18). Containers of dry powdered hazardous materials, (e.g., Class 3 fungal agents in the spore phase of growth) should be opened only in a Biological Safety Cabinet (6,14).

7. When the neck of an ampoule containing liquid is broken after nicking with a file, the snapping action creates aerosols. The following methods have been recommended:

   (i) After nicking the ampoule with a file, wrap the ampoule in disinfectant-wetted cotton before breaking. Wear gloves (2).

   (ii) The bottom of the ampoule should be held in several layers of tissue paper to protect the hands, and a file mark made at the neck. A hot glass rod should be carefully applied to the mark. The glass will crack, allowing air to enter the ampoule and equalize the pressures. After a few seconds the ampoule should be wrapped in a few layers of tissue and broken along the crack. The tissues and ampoule neck can then be discarded into disinfectant, and the contents of the ampoule removed with a syringe. If

the ampoule contains dried cultures, about 0.5 $cm^3$ of broth should be added slowly to avoid blowing dried material out. The contents may then be mixed without bubbling and withdrawn into a culture tube (8).

(iii) The researcher uses an intense, but tiny, gas-oxygen flame and heats the tip of the hard glass ampoule until the expanding internal air pressure blows a bubble. After allowing this to cool, he breaks the bubble while holding it in a large low temperature flame; this immediately incinerates any infectious dust which may come from the ampoule when the glass is broken (16). Preliminary practice with a simulant ampoule of the same type actually in use is necessary to develop a technique that will not cause explosion of the ampoule.

(iv) A simple device has been recommended consisting of a sleeve of rubber tubing into which the ampoule is inserted before it is broken (17,18).

D. Centrifuging

1. A safety centrifuge cabinet or safety centrifuge cup (3,7, 8,14,22) may be used to house or safeguard all centrifuging of infectious substances. When bench type centrifuges are used in a Biological Safety Cabinet, the glove panel should

be in place with the glove ports covered. The centrifuge operation creates air currents that may cause escape of agent from an open cabinet (2,3,4,13).

2. In some situations, in the absence of O-ring cap sealed trunnion cups, specimens can be enclosed in sealed plastic bags before centrifugation (12).
3. Before centrifuging, inspect tubes for cracks, inspect the inside of the trunnion cup for rough walls caused by erosion or adhering matter, and carefully remove bits of glass from the rubber cushion (4,10).
4. A germicidal solution should be added between the tube and trunnion cup to disinfect the materials in case of accidential breakage. This practice also provides an excellent cushion against shocks that might otherwise break the tube (4,10).
5. Avoid decanting centrifuge tubes. If you must do so, afterwards wipe off the outer rim with a disinfectant; otherwise the infectious fluid will spin off as an aerosol (4,10).
6. Avoid filling the tube to the point that the rim, cap or cotton plug ever becomes wet with culture (4,10).
7. Screw caps, or caps which fit over the rim outside the centrifuge tube are safer than plug-in closures. Some fluid usually collects between a plug-in closure and the rim of the tube. Even screw-capped bottles are not without risk,

however; if the rim is soiled some fluid will escape down the outside of the tube. Screw-capped bottles may jam in the bucket, and removing them is hazardous. Propping such bottles higher in the bucket with additional rubber buffers is mechanically unsound (8).

8. Kitchen foil is often used to cap centrifuge tubes. This creates more risk than the screw cap. Foil caps often become detached in handling and centrifuging (8).
9. The balancing of buckets is often mismanaged. Care must be taken to ensure that matched sets of trunnions, buckets and plastic inserts do not become mixed. If the components are not inscribed with their weights by the manufacturer, colored stains can be applied to avoid confusion. When the tubes are balanced, the buckets, trunnions and inserts should be included in the procedure; and care must be taken to ensure that the centers of gravity of the tubes are equidistant from the axis of rotation. To illustrate the importance of this, two identical tubes containing 20g of mercury and 20g of water respectively will balance perfectly on the scales; but their performance in motion is totally different, leading to violent vibration with all its attendant hazards (5).
10. Fill and open centrifuge tubes or trunnion cups in a Biological Safety Cabinet (10).

E. High-Speed Centrifuges (22)

1. In high-speed centrifuges the bowl is connected to a vacuum pump. If there is a breakage or accidental dispersion of infected particles the pump and the oil in it will become contaminated. A high efficiency filter should be placed between the centrifuge and the pump (8).

2. High speed rotor heads are prone to metal fatigue, and where there is a chance that they may be used on more than one machine each rotor should be accompanied by its own log book indicating the number of hours run at top or de-rated speeds. Failure to observe this precaution can result in dangerous and expensive disintegration. Frequent inspection, cleaning and drying are important to ensure absence of corrosion or other traumata which may lead to creeping cracks. Rubber O-rings and tube closures must be examined for deterioration and be kept lubricated with the material recommended by the makers. Where tubes of different materials are provided (e.g., celluloid, polypropylene, stainless steel), care must be taken that the tube closures designed specifically for the type of tube in use are employed. These caps are often similar in appearance, but are prone to leakage if applied to tubes of the wrong material. When

properly designed tubes and rotors are well maintained and handled, leaking should never occur (5).

3. Cleaning and disinfection of tubes, rotors and other components requires considerable care. It is unfortunate that no single process is suitable for all items, and the various manufacturers' recommendations must be followed meticulously if fatigue, distortion and corrosion are to be avoided. This is not the place to catalogue recommended methods, but one less well appreciated fact is worthy of mention. Celluloid (cellulose nitrate) centrifuge tubes are not only highly inflammable and prone to shrinkage with age and distortion on boiling, but can behave as high explosive in an autoclave (5). Large-scale zonal centrifugation requires special attention (11).

F. Blenders, Ultrasonic Disintegrators, Colloid Mills, Ball Mills, Jet Mills, Grinders, Mortar and Pestle

All these devices release considerable aerosols during their operation. For maximum protection to the operator during the blending of infectious materials, the following practices should be observed:

1. Operate blending and cell-disruption and grinding equipment in a Biological Safety Cabinet (9).

2. Use safety blenders designed to prevent leakage from the rotor bearing at the bottom of the bowl (9).
3. In the absence of a leak-proof rotor, inspect the rotor bearing at the bottom of the blender bowl for leakage prior to operation. Test it in a preliminary run with sterile saline or methylene blue solution prior to use with infected material (9).
4. Sterilize the device and residual infectious contents promptly after use. Use a towel moistened with disinfectant over the top of the blender (9).
5. Glass blender bowls are undesirable for use with infectious material because of potential breakage. If used, they should be covered with a polypropylene jar to prevent dispersal of glass (8).
6. A new machine, the Colworth Stomacker (England), in which material is homogenized in a plastic bag in a closed container, would appear to be safer than some of the other blenders (8).
7. A heat-sealed flexible plastic film enclosure for a grinder or blender can be used, but it must be opened in a Biological Safety Cabinet (7).
8. Blender bowls sometimes require supplemental cooling to prevent destruction of the bearings and to minimize thermal efforts on the product (7).

9. Before opening the safety blender bowl, permit the blender to rest for at least one minute to allow settling of the aerosol cloud.

10. Clinical or other laboratories handling human blood should be aware of the aerosols produced by the micro-haematocrit centrifuge, the autoanalyzer stirrer, and the microtonometer, inasmuch as it seems that airborne transmission of infectious hepatitis may occur in the laboratory (20).

G. Miscellaneous Precautions and Recommendations

1. Water baths and Warburg baths used to inactivate, incubate, or test infectious substances should contain a disinfectant. For cold water baths, 70% propylene glycol is recommended (4,10).

2. Deepfreeze, liquid nitrogen, and dry ice chests and refrigerators should be checked and cleaned out periodically to remove any broken ampoules, tubes, etc., containing infectious material, and decontaminated. Use rubber gloves and respiratory protection during this cleaning. All infectious or toxic material stored in refrigerators or deepfreezes should be properly labelled. Security measures should be commensurate with the hazards (4,10,21).

3. Freeze-dried culture ampoules should always be opened in a Biological Safety Cabinet. The ampoule should be wrapped in a disinfectant-soaked swab before breaking it open to minimize the risk of cutting the hands, and to a lesser extent of releasing aerosol of dried material. Whenever possible, ampoules should be filled with dry nitrogen after freeze-drying, thus avoiding implosion that may occur during the sealing as well as opening of evacuated ampoules. The whole process of freeze-drying itself should be performed in a Biological Safety Cabinet. Filtration of the effluent air from the vacuum pump is desirable either up (preferably), or down stream of the pump (5).
4. Ensure that all virulent fluid cultures or viable powdered infectious materials in glass vessels are transported, incubated, and stored in easily handled, nonbreakable leak-proof containers that are large enough to contain all the fluid or powder in case of leakage or breakage of the glass vessel (4,10).
5. All inoculated petri plates or other inoculated solid media should be transported and incubated in leak-proof pans or leak-proof containers (4,10).
6. Care must be exercised in the use of membrane filters to obtain sterile filtrates of infectious materials. Because

of the fragility of the membrane and other factors, such filtrates cannot be handled as noninfectious until culture or other tests have proved their sterility (4,10).

7. Shaking machines should be examined carefully for potential breakage of flasks or other containers being shaken. Screw capped durable plastic or heavy walled glass flasks should be used. These should be securely fastened to the shaker platform. An additional precaution would be to enclose the flask in a plastic bag with or without an absorbent material.
8. No person should work alone on an extremely hazardous operation (4,10).

## IV. Personal Hygiene, Habits, and Practices

Personal hygienic practices in the laboratory are directed, in most part, toward the prevention of occupationally acquired physical injury or disease. To a less obvious extent, they can raise the quality of the laboratory work by reducing the possibilities for contamination of experimental materials. The reasons for many of the recommended precautions and practices are obvious, but, in some instances, amplification will permit a better review of the applicability to any one specific laboratory.

Consequently, what might be forbidden in one laboratory might be only discouraged in another, and be permissible in a third. Nevertheless, adherence to safe practices that become habitual, even when seemingly not essential, provides a margin of safety in situations where the hazard is unrecognized. The history of occupational injury is replete with examples of hazards unrecognized until too late. The following guidelines, recommendations, and comments are presented with this in mind:

1. Food, candy, gum, and beverages for human consumption will be stored and consumed only outside the laboratory (5,10).
2. Foot-operated drinking fountains should be the sole source of water for drinking by human occupants of the laboratory (27).
3. Smoking is not permitted in the laboratory or animal quarters. Cigarettes, pipes, and tobacco will be kept only in clean areas (5,10,26).

4. Shaving and brushing of teeth are not permitted in the laboratory. Razors, toothbrushes, toiletry supplies, and cosmetics are permissible only in clean change rooms or other clean areas, and should never be used until after showering or thorough washing of the face and hands (27).
5. A beard may be undesirable in the laboratory in the presence of actual or potential airborne contamination, because it retains particulate contamination more persistently than clean-shaven skin. A clean-shaven face is essential to the adequate facial fit of a face mask or respirator when the work requires respiratory protection (10,27,31).
6. Develop the habit of keeping hands away from mouth, nose, eyes, face, and hair. This may prevent self-inoculation (10,27).
7. For product protection, persons with long hair should wear a suitable hair net or head cover that can be decontaminated. This has long been a requirement in hospital operating rooms and in the manufacture of biological pharmaceutical products. A head cover also will protect the hair from fluid splashes, from swinging into Bunsen flames and petri dishes, and will reduce facial contamination caused by habitual repetitive manual adjustment of the hair (5).

8. Long-flowing hair and loose-flapping clothing are dangerous in the presence of open flame or moving machinery. Rings and wrist watches also are a mechanical hazard during operation of some types of machines (5,10).
9. Contact lenses do not provide eye protection. The capillary space between the contact lenses and the cornea may trap any material present on the surface of the eye. Caustic chemicals trapped in this space cannot be washed off the surface of the the cornea. If the material in the eye is painful or the contact lens is displaced, muscle spasms will make it very difficult, if not impossible, to remove the lens. For this reason, contact lenses must not be worn by persons exposed to caustic chemicals unless safety glasses with side shields, goggles, or plastic face masks are also worn to provide full protection. It is the responsibility of supervisors to identify employees who wear contact lenses (25,26).
10. Personal items, such as coats, hats, storm rubbers or overshoes, umbrellas, purses, etc., do not belong in the laboratory. These articles should be kept elsewhere (25).
11. Plants, cut flowers, an aquarium, and pets of any kind are undesirable sources of yeast, molds, and other potential microbial contaminants of biological experimental materials (25).

12. Books and journals returnable to the institutional library should be used only in the clean areas as much as possible (10,27).
13. When change rooms with showers are provided, the employer should furnish skin lotion (27).
14. When employees are subject to potential occupational infection, the shower and/or face/hand-washing facilities should be provided with germicidal soap (8,27).
15. Personal cloth handkerchiefs should not be used in the laboratory. Cleansing tissue should be available instead.
16. Hand washing for personal protection:

    (i) This should be done promptly after removing protective gloves. Tests show it is not unusual for microbial or chemical contamination to be present despite use of gloves, due to unrecognized small holes, abrasions, tears, or entry at the wrist.

    (ii) Throughout the day, at intervals dictated by the nature of the work, the hands should be washed. Presence of a wrist watch discourages adequate washing of the wrist (10,25).

    (iii) Hands should be washed after removing soiled protective clothing, before leaving the laboratory area, before eating, and before smoking. The provision of hand cream by the employer encourages these practices (5,8,10).

(iv) A disinfectant wash or dip may be desirable in some cases, but its use must not be carried to the point of causing roughening, desiccation or sensitization of the skin.

17. Anyone with a fresh or healing cut, abrasion, or skin lesion should not work with infective material unless the injured area is completely protected (8,25).

18. Persons vaccinated for smallpox may be shedders of vaccinia virus during the phase of cutaneous reaction. Therefore, vaccination requires permission of the appropriate supervisor, because two weeks' absence may be necessary before returning to work with normal cell cultures or with susceptible animals, especially the normal mouse colony (25).

19. The surgeon's mask of gauze or filter paper is of little value for personal respiratory protection (29). It is designed to prevent escape of droplets from the nose or mouth (23G). If biohazards demand respiratory protection, then nothing but a full face respirator or ventilated hood will suffice. A half-mask respirator does not protect the eyes, which are an un-evaluated avenue of infection through the conjunctiva and the nasolacrimal duct (5,8).

20. Nonspecific contamination by environmental organisms from humans, animals, equipment, containers for specimens or

supplies, and outside air is a complication that may affect or invalidate the results of an experiment. The human sources of this contamination are evaluated as follows:

(i) Sneezing, coughing and talking (23A,24A). Sneezing, variously reported to generate as many as 32,000 or 1,000,000 droplets below 100 microns in diameter; coughing, which produces fewer and larger droplets; and talking, which has been reported to average only 250 droplets when speaking 100 words, show great differences between persons in regard to the number of microorganisms aerosolized. As a general rule, it may be said that these actions by normal healthy persons may play a less important role in transmission of airborne infection to humans or experimental materials than does liberation of microorganisms from human skin.

(ii) Dispersal of bacteria from human skin. There is a tremendous variation in the number of bacteria shed from the skin by a clothed subject. For instance, in one study, the number varied from 6,000 to 60,000 per minute (23C). These bacteria were released on skin scales which were of a size that could penetrate the coarse fabric used for the laboratory and surgical clothing in the test (23D). Dispersal of skin bacteria was several times greater from below the waist than from upper parts of the body (24D). Effective reduction is accomplished

by use of closely-woven or impervious clothing fitted tightly at the neck, wrists, and ankles to prevent the clothing from acting as a bellows that disperses air carrying skin scales laden with bacteria (23B). Such clothing sometimes is too warm to work in. It was found that a significant reduction in dispersal of bacteria occurred with the wearing of close-fitting and closely-woven underpants beneath the usual laboratory clothing (23D). The purpose of this summary is to alert laboratory personnel to the existence of this source of contamination (9).

(iii) Prolific dispersal of bacteria occurs from infected abrasions, small pustules, boils, and skin disease (23F,24B). Washing the lesions with germicidal soap will greatly decrease the number of organisms on the skin and dispersal into the air. Healthy nasal carriers who generate aerosolized staphylococci usually can be identified by the presence of heavy contamination of their fingers, face, and hair (23E). This point may be useful in investigating the source of staphylococcal contamination of cell lines.

(iv) Footwear. In moderate and high risk situations, shoes reserved for only laboratory use have been recommended as a precaution against transporting spilled infectious agents outside the laboratory. However, in experiments during which reduction of potential contamination of experimental materials is important, laboratory-only shoes can reduce the microbial load brought into the laboratory each day by street shoes. Shoes are efficient transporters. In one study, there were 4 to 850 times as many bacteria per square centimeter on the laboratory footwear as on the floor itself (30).

V. Care and Use of Laboratory Animals (10,32-37)

A. Care and Handling

1. Special attention must be given to the humane treatment of all laboratory animals in accordance with the Animal Welfare Act of 1970. The implementing rules and regulations appear in the Code of Federal Regulations (CFR) Title 9, Chapter 1, Subchapter A, parts 1, 2, 3. Recommended provisions and practices that meet the requirements of the Act have been published by the U.S. Public Health Service (32).

2. There are specific minimum requirements (33) concerning the caging, feeding, watering, and sanitation for dogs, cats, guinea pigs, hamsters, rabbits, and nonhuman primates. To meet these requirements, the animal room supervisor must have a copy of 9 CFR Chapter 1, Subchapter A, Parts 1, 2, 3.

3. Each laboratory should establish procedures to ensure the use of animals that are free of disease prejudicial to the proposed experiments and free from carriers of disease or vectors, such as ectoparasites, which endanger other experimental animals or personnel (10).

B. Cages Housing Infected Animals (10)

1. Careful handling procedures should be employed to minimize the dissemination of dust from cage refuse and animals.

2. Cages should be sterilized by autoclaving. Refuse, bowls and watering devices should remain in the cage during sterilization.
3. All watering devices should be of the "non-drip" type.
4. Cages should be examined each morning and at each feeding time so that dead animals can be removed.
5. Heavy gloves should be worn when feeding, watering, handling, or removing infected animals. Bare hands should NEVER be placed in the cage to move any object therein.
6. When animals are to be injected with biohazardous material, the animal caretaker should wear protective gloves and the laboratory workers should wear surgeons gloves. Animals should be properly restrained to avoid accidents that might result in disseminating biohazardous material, as well as to prevent injury to the animal and to personnel.
7. Animals exposed to biohazardous aerosols should be housed in ventilated cages, in gas-tight cabinet systems, or in rooms designed for protection of personnel by use of ventilated suits.
8. Animals inoculated by means other than by aerosols should be housed in equipment suitable for the level of risk involved.
9. Infected animals to be transferred between buildings should be placed in ventilated cages or other aerosol-proof containers.

10. The oversize canine teeth of large monkeys present a particular biting hazard; these are important in the potential transmission of naturally-occurring, and very dangerous, monkey virus infections. Such teeth should be blunted or surgically removed by a veterinarian.
11. Presently available epidemiological evidence indicates that infectious hepatitis may be transmitted from non-human primates (typically chimpanzees) to man. Newly imported animals may be naturally infected with this disease, and persons in close contact with such animals may become infected. After six months residence in this country, chimpanzees apparently no longer transmit the disease. A record should be maintained for each newly imported animal. A sign should be posted at rooms housing these animals to warn that the animals are potentially infectious.

C. General Guidelines that Apply to Animal Room Maintenance (10)

1. Doors to animal rooms should be kept closed at all times except for necessary entrance and exit.
2. Unauthorized persons should not be permitted to enter animal rooms.
3. A container of disinfectant should be kept in each animal room for disinfecting gloves and hands, and for general decontamination, even though no infectious animals are present. Hands, floors, walls, and cage racks should be

washed with an approved disinfectant at the recommended strength as frequently as the supervisor directs.

4. Floor drains in animal rooms, as well as floor drains throughout the building should be flooded with water or disinfectant periodically to prevent backup of sewer gases.
5. Shavings or other refuse on floors should not be washed down the floor drain because such refuse clogs the sewer lines.
6. An insect and rodent control program should be maintained in all animal rooms and in animal food storage areas.
7. Special care should be taken to prevent live animals, especially mice, from finding their way into disposable trash.

D. Necropsy Rules for Infected Animals (10)

1. Necropsy of infected animals should be carried out by trained personnel in Biological Safety Cabinets with the hinged glass panel down. The glove port panel with or without attached gloves, and a respirator should be used at the discretion of the supervisor.
2. Surgeons gowns should be worn over laboratory clothing during necropsies.
3. Rubber gloves should be worn when performing necropsies.
4. The fur of the animal should be wetted with a suitable disinfectant.

5. Small animals should be pinned down or fastened on wood or metal in a metal tray.
6. Upon completion of necropsy, all potentially biohazardous material should be placed in suitable containers and sterilized immediately.
7. Contaminated instruments should be placed in a horizontal bath containing a suitable disinfectant.
8. The inside of the Biological Safety Cabinets and other potentially contaminated surfaces should be disinfected with a suitable germicide.
9. Grossly contaminated rubber gloves should be cleaned in disinfectant before removal from the hands, preparatory to sterilization.
10. Dead animals should be placed in proper leak-proof containers, autoclaved and properly tagged before being placed outside for removal and incineration.

## VI. Decontamination and Disposal (7,10,38-42)

### A. Introduction

Available data on the efficacy of various decontaminants for etiologic agents indicate that no major surprises will be forthcoming regarding the susceptibility of organisms containing recombinant DNA molecules. In the absence of adequate information, tests to determine the efficacy of candidate decontaminants should be conducted with the specific agent of interest. The goal of decontamination is not only the protection of personnel and the environment from exposure to infectious agents, but also the prevention of contamination of experimental materials by a variable, persistent, and unwanted background of microorganisms. This additional factor should be considered in selecting decontamination materials and methods.

### B. Decontamination Methods

Physical and chemical means of decontamination fall into four main categories: Heat; Liquid Decontaminants; Vapors and Gases; and UV Radiation.

1. *Heat.* The application of heat, either moist or dry, is recommended as the most effective method of sterilization. Steam at 121 C under pressure in the autoclave is the most convenient method of rapidly achieving sterility. Dry heat at 160 to 170 C for periods of 2 to 4 hours is suitable for destruction of viable agents on impermeable nonorganic material such as glass, but is not reliable in even shallow layers of

organic or inorganic material that can act as insulation. Incineration is another use of heat in the decontamination of microorganisms and also serves as an efficient means for disposal.

2. *Liquid Decontaminants.* In general, the liquid decontaminants find their most practical use in surface decontamination and, at sufficient concentration, as decontaminants of liquid wastes for final disposal in sanitary sewer systems.

There are many misconceptions concerning the use of liquid decontaminants. This is due largely to a characteristic capacity of such liquids to perform dramatically in the test tube and to fail miserably in a practical situation. Such failures often occur because proper consideration was not given to such factors as temperature, time of contact, pH, concentration, and the presence and state of dispersion, penetrability and reactivity of organic material at the site of application. Small variations in the above factors may make large differences in effectiveness of decontamination. For this reason, even when used under highly favorable conditions, complete reliance should not be placed on liquid decontaminants when the end result must be sterility.

There are many liquid decontaminants available under a wide variety of trade names. In general, these can be categorized as halogens, acids or alkalies, heavy metal salts, quaternary ammonium compounds, phenolic compounds, aldehydes,

ketones, alcohols and amines. Unfortunately, the more active the decontaminant the more likely it is that the decontaminant will possess undesirable characteristics, such as the possession of corrosive properties. None is equally useful or effective under all conditions.

3. *Vapors and Gases.* A variety of vapors and gases possess decontamination properties. The most useful of these are formaldehyde and ethylene oxide. When these can be employed in closed systems and under controlled conditions of temperature and humidity, excellent decontamination can result. Vapor and gas decontaminants are primarily useful in decontaminating: (i) Biological Safety Cabinets and associated effluent air-handling systems and air filters; (ii) bulky or stationary equipment that resists penetration by liquid surface decontaminants; (iii) instruments and optics that might be damaged by other decontamination methods; and (iv) rooms and buildings and associated airhandling systems.

4. *Radiation.* The usefulness of ultraviolet (UV) irradiation as a decontaminant is limited by its low penetrating power. No information is available regarding the effectiveness of UV irradiation for decontaminating microorganisms containing recombinant DNA molecules. Dependence on UV must be based on the results of experiments imitating particular anticipated environmental conditions and applications. Ultraviolet light is generally of limited application and is primarily useful in air locks and

animal holding areas for controlling low levels of airborne contaminants.

No one procedure or material will solve all decontamination problems. The only method of assuring the efficacy of selected methodologies is to critically examine the results obtained in practical tests with the microorganism(s) of interest.

C. Laboratory Spills

A troublesome problem that may occur in the laboratory is the decontamination of an overt biological spill. The occurrence of a spill poses less of a problem if it occurs in a Biological Safety Cabinet provided splattering to the outside of the cabinet does not occur. Direct application of concentrated liquid decontaminant and a thorough wipe down of the internal surfaces of such cabinetry will usually be effective for decontaminating the work zone but gaseous decontaminants would be required to rid the interior sections of the cabinet of contaminants. Each researcher must realize that in the event of an overt accident, research materials such as tissue cultures, media, and animals within such cabinets may well be lost to the experiment.

The greater problem arises if the incident occurs in the open laboratory. All laboratory protocols should be designed to prevent such occurrences. The first action in the event of an overt laboratory spill is evacuation of the affected area to minimize the exposure of personnel involved. Next, the spill area must be isolated to prevent exposure of personnel and experimental

materials beyond those involved in the immediate area of the spill. The procedures adopted must be rapidly effective and must not create additional aerosol or foster mechanical transfer of materials to unaffected areas. Personnel carrying out the procedures must be provided with protective clothing and equipment, including respiratory protection. Consideration must be given to the safe disposal of all materials and liquids resulting from cleanup procedures. Reentry of personnel to the area should be avoided until it can be reasonably established that the area has been effectively decontaminated. Further specific details are provided in Section VIII.

D. Disposal

Decontamination and disposal in infectious disease laboratories are closely interrelated acts in which decontamination constitutes the introductory phase of disposal. All materials and equipment used in research on recombinant DNA molecules will ultimately be disposed of; however, in the sense of daily use, only a portion of these will require actual removal from the laboratory complex or on-site destruction. The remainder will be recycled for use either within the same laboratory or in other laboratories that may or may not engage in DNA recombinant research. Examples of the latter that immediately come to mind are: reusable laboratory glassware, instruments used in necropsy of infected animals, and laboratory clothing. Disposal should therefore be interpreted in the broadest sense of the word, rather than in the restrictive sense of dealing solely with a destructive process.

The principal questions to be answered prior to disposal of any objects or materials from laboratories dealing with potentially infectious microorganisms or animal tissues are:

1. Have the objects or materials been effectively decontaminated by an approved procedure?
2. If not, have the objects or materials been packaged in an approved manner for immediate on-site incineration or transfer to another laboratory?
3. Does disposal of the decontaminated objects or materials involve any additional potential hazards, biological or otherwise, to personnel either:

   (i) those carrying out the immediate disposal procedures or

   (ii) those who might come into contact with the objects or materials outside the laboratory complex?

Laboratory materials requiring disposal will normally occur as liquid, solid, and animal room wastes. The volume of these can become a major problem when there is the requirement that all wastes be decontaminated prior to disposal. It is most evident that a significant portion of this problem can be eliminated if the kinds of materials initally entering the laboratory are reduced. In any case, and wherever possible, materials not essential to the research should be retained in the non-research areas for disposal by conventional methods. Examples are the packaging materials in which goods are delivered, disposable carton-cages

for transport of animals, and large carboys or tanks of fluids which can be left outside and drawn from as required. Reduction of this bulk will free autoclaves and other decontamination and disposal processes within the laboratory for the more rapid and efficient handling of materials known to be contaminated.

Inevitably, disposal of materials raises the question, "How can we be sure that the materials have been treated adequately to assure that their disposal does not constitute a hazard?" In the small laboratory, the problem is often solved by requiring that each investigator decontaminate all contaminated materials not of immediate use at the end of each day and place them in suitable containers for routine disposal. In larger laboratories where the mass of materials for disposal becomes much greater and sterilization and decontamination bottlenecks occur, materials handling and disposal will likely be the chore of personnel not engaged in the actual research. In either situation, a case can be made for establishing a positive method of designating the state of materials to be disposed of. This may consist of a tagging system stating that the materials are either sterile or contaminated.

Disposal of materials from the laboratory and animal holding areas will be required for research projects ranging in size from an individual researcher to those involving large numbers of researchers of many disciplines. Procedures and facilities to accomplish this will range from the simplest to the most elaborate. The primary consideration in any of these is to dispel the notion that laboratory wastes can be disposed of in the same manner and

with as little thought as household wastes. Selection and enforcement of safe procedures for disposal of laboratory materials are of no less importance than the consideration given to any other methodology for the accomplishment of research objectives.

Materials of dissimilar nature will be common in laboratories studying recombinant DNA molecules. Examples are combinations of common flammable solvents, chemical carcinogens, radioactive isotopes, and concentrated viruses or nucleic acids. These may require input from a number of disciplines in arriving at the most practical approach for their decontamination.

## E. Characteristics of Chemical Decontaminants in Common Use in Laboratory Operations

Every person actively working with viable microorganisms, no matter how remote the field of specialization, will, from time to time, find it necessary to decontaminate by chemical methods work areas and materials, equipment, and specialized instruments. Chemical decontamination is necessary because the use of pressurized steam, the most rapid and reliable method of sterilization, is not normally feasible for decontaminating large spaces, surfaces, and stationary equipment. Moreover, high temperatures and moisture often damage delicate instruments, particularly those having complex optical and electronic components.

Chemicals with decontaminant properties are, for the most part, available as powders, crystals, and liquid concentrates. These may be

added to tap water for application as surface decontaminants, and some, when added in sufficient quantity, find use as decontaminants of bulk liquid wastes. Chemical decontaminants that are gaseous at room temperatures are useful as space-penetrating decontaminants. Others become gases at reasonably elevated temperatures and can act as either aqueous surface or gaseous space-penetrating decontaminants.

Inactivation of microorganisms by chemical decontaminants may occur in one or more of the following ways:

1. Coagulation and denaturation of protein
2. Lysis
3. Binding to enzymes, or inactivation of an essential enzyme by either oxidation, binding, or destruction of enzyme substrate.

The relative resistance to the action of chemical decontaminants can be substantially altered by such factors as: concentration of active ingredient, duration of contact, pH, temperature, humidity, and presence of extrinsic organic matter. Depending upon how these factors are manipulated, the degree of success achieved with chemical decontaminants may range from minimal inactivation of target microorganisms to an indicated sterility within the limits of sensitivity of the assay systems employed.

There are dozens of decontaminants available under a wide variety of trade names. In general, these decontaminants can be classified as halogens, acids or alkalies, heavy metal salts, quaternary ammonium compounds,

phenolic compounds, aldehydes, ketones, alcohols, and amines. Unfortunately, the more active the decontaminant the more likely it will possess undesirable characteristics. For example, peracetic acid is a fast-acting, universal decontaminant. However, in the concentrated state it is a hazardous compound that can readily decompose with explosive violence. When diluted for use, it has a short half-life, produces strong, pungent, irritating odors, and is extremely corrosive to metals. Nevertheless, it is such an outstanding decontaminant that it is commonly used in germ-free animal studies despite these undesirable characteristics.

The halogens are probably the second most active group of decontaminants. Chlorine, iodine, bromine, and fluorine will rapidly kill bacterial spores, viruses, rickettsiae, and fungi. These decontaminants are effective over a wide range of temperatures. In fact, chlorine has been shown to be effective at -40 F. (On the other hand, phenols and formaldehyde have high temperature coefficients). The halogens have several undesirable features. They readily combine with protein, so that an excess of the halogen must be used if proteins are present. Also, the halogens are relatively unstable so that fresh solutions must be prepared at frequent intervals. Finally, the halogens corrode metals. A number of manufacturers of decontaminants have treated the halogens to remove some of the undesirable features. For example, sodium hypochlorite reacts with p-toluenesulfonamide to form Chloramine T, and iodine reacts with certain surface-active agents to form the popular iodophors. These "tamed" halogens are stable, non-toxic, odorless, and relatively noncorrosive to metals. However, the halogens are highly reactive elements, and, because they are reactive they are good germi-

cides. When a halogen acts as a decontaminant, free halogen is the effective agent. Raising the pH or combining the halogen with other compounds to decrease the corrosive effect will also decrease the germicidal power. A trade-off situation occurs.

Ineffectiveness of a decontaminant is due primarily to the failure of the decontaminant to contact the microorganisms rather than failure of the decontaminant to act. If one places an item in a liquid decontaminant, one can see that the item is covered with tiny bubbles. Of course, the area under the bubbles is dry, and microorganisms in these dry areas will not be affected by the decontaminant. Also, if there are spots of grease, rust or dirt on the object, microorganisms under these protective coatings will not be contacted by the decontaminant. Scrubbing an item when immersed in a decontaminant is helpful, and a decontaminant should have, and most do have, incorporated surface-active agents.

F. Properties of Some Common Decontaminants

1. *Alcohol.* Ethyl or isopropyl alcohol in a concentration of 70-85% by weight is often used. Alcohols denature proteins and are somewhat slow in their germicidal action. However, they are effective decontaminants against lipid-containing viruses.

2. *Ether and Chloroform.* These compounds are not ordinarily used as decontaminants, but they do demonstrate the fact that lipid-containing viruses are inactivated by these organic solvents, whereas non-lipid-containing viruses are quite resistant.

3. *Formaldehyde.* Formaldehyde for use as a decontaminant is usually marketed as a solution of about 37% concentration referred to as formalin or as a solid polymerized compound called paraformaldehyde. Formaldehyde in a concentration of 5% active ingredient is an effective liquid decontaminant. It loses considerable activity at refrigeration temperatures and the pungent, irritating odors make formaldehyde solutions difficult to use in the laboratory. Formaldehyde vapor generated from formaldehyde solution is an effective space decontaminant for decontaminating rooms or buildings, but in the vapor state with water it tends to polymerize out on surfaces to form paraformaldehyde, which is persistent and unpleasant. Formaldehyde gas can be liberated by heating paraformaldehyde to depolymerize it. In the absence of high moisture content in the air, formaldehyde released in the gaseous state forms less polymerized residues on surfaces and less time is required to clear treated areas of fumes than formaldehyde released in the vapor state.

4. *Phenol.* Phenol itself is not often used as a decontaminant. The odor is somewhat unpleasant and a sticky, gummy residue remains on treated surfaces. This is especially true during steam sterilization. Although phenol itself may not be in widespread use, phenol homologs and phenolic compounds are basic to a number of popular decontaminants. The phenolic compounds are effective decontaminants against some viruses,

rickettsiae, fungi and vegetative bacteria. The phenolics are not effective in ordinary usage against bacterial spores.

5. *Quaternary Ammonium Compounds or Quats.* After 30 years of testing and use, there is still a considerable controversy about the efficacy of the Quats as decontaminants. These cationic detergents are strongly surface-active and are effective against lipid-containing viruses. The Quats will attach to protein so that dilute solutions of Quats will quickly lose effectiveness in the presence of proteins. The Quats tend to clump microorganisms and are neutralized by anionic detergents, such as soap. The Quats have the advantages of being nontoxic, odorless, nonstaining, noncorrosive to metals, stable, and inexpensive.

6. *Chlorine.* This halogen is a universal decontaminant active against all microorganisms, including bacterial spores. Chlorine combines with protein and rapidly decreases in concentration in its presence. Free, available chlorine is an active element. It is a strong oxidizing agent, corrosive to metals. Chlorine solutions will gradually lose strength so that fresh solutions must be prepared frequently. Sodium hypochlorite is usually used as a base for chlorine decontaminants. An excellent decontaminant can be prepared from household or laundry bleach. These bleaches usually contain 5.25 percent available chlorine or 52,500 ppm. If one dilutes them 1 to 100, the solution will contain 525 ppm of available

chlorine, and, if a nonionic detergent such as Naccanol is added in a concentration of about 0.7 percent, a very good decontaminant is created.

7. *Iodine.* The characteristics of chlorine and iodine are similar. One of the most popular groups of decontaminants used in the laboratory is the iodophors, and Wescodyne is perhaps the most popular. The range of dilution of Wescodyne recommended by the manufacturer is 1 oz. in 5 gal. of water giving 25 ppm of available iodine to 3 oz. in 5 gal. giving 75 ppm. At 75 ppm, the concentration of free iodine is .0075 percent. This small amount can be rapidly taken up by any extraneous protein present. Clean surfaces or clear water can be effectively treated by 75 ppm available iodine, but difficulties may be experienced if any appreciable amount of protein is present. For bacterial spores, a dilution of 1 to 40 giving 750 ppm is recommended by the manufacturer. For washing the hands, it is recommended that Wescodyne be diluted 1 to 10 or 10% in 50% ethyl alcohol (a reasonably good decontaminant itself) which will give 1,600 ppm of available iodine, at which concentration relatively rapid inactivation of any and all microorganisms will occur.

G. Vapors and Gases

The use of formaldehyde as a vapor or gas has already been discussed. Other chemical decontaminants which have been used this way included ethylene oxide, peracetic acid, beta-propiolactone (BPL), methyl bromide, and ethylene

amine. When these can be used in closed systems and under controlled conditions of temperature and humidity, excellent decontamination can be obtained. Residues from ethylene oxide must be removed by aeration; but otherwise it is convenient to use, versatile, and noncorrosive. Peracetic acid is corrosive for metals and rubber. BPL in the vapor form acts rapidly against bacteria, rickettsiae, and viruses. It has a half-life of 3.5 hours when mixed with water, is easily neutralized with water, and lends itself to removal by aeration. The National Institutes of Health does not recommend BPL as a decontaminant because it has been identified as a suspect carcinogen.

H. Residual Action of Decontaminants

As noted in the preceding discussion of decontaminant properties, many of the chemical decontaminants often have residual properties that may be considered a desirable feature in terms of aiding in the control of background contamination. One is cautioned, however, to consider residual properties carefully. Ethylene oxide used to sterilize laboratory shoes can leave residues which cause skin irritation. Animal cell cultures, as well as viruses of interest, are also inhibited or inactivated by decontaminants persisting after routine cleaning procedures. Therefore, reusable items that are routinely held in liquid decontaminant prior to autoclaving and cleaning should receive particular attention in rinse cycles. Similarly, during general area decontamination with gases or vapors, it may be necessary to protect new and used clean items by removing them from the area or by enclosing them in gastight bags or by insuring adequate aeration following decontamination.

I. Selecting Chemical Decontaminants for Research on Recombinant DNA Molecules

No single chemical decontaminant or method will be effective or practical for all situations in which decontamination is required. Selection of chemical decontaminants and procedures must be preceded by practical consideration of the purposes for the decontamination and the interacting factors that will ultimately determine how that purpose is to be achieved. Selection of any given procedure will be influenced by the information derived from answers to the following questions:

1. What is the target microorganism(s)?
2. What decontaminants in what form are known to, or can be expected to, inactivate the target microorganism(s)?
3. What degree of inactivation is required?
4. In what menstruum is the microorganism suspended; i.e., simple or complex, on solid or porous surfaces, and/or airborne?
5. What is the highest concentration of cells anticipated to be encountered?
6. Can the decontaminant either as an aqueous solution, a vapor, or a gas reasonably be expected to contact the microorganisms, and can effective duration of contact be maintained?
7. What restrictions apply with respect to compatibility of materials?

8. Does the anticipated use situation require immediate availability of an effective concentration of the decontaminant or will sufficient time be available for preparation of the working concentration shortly before its anticipated use?

The primary target of decontamination in the infectious disease laboratory is the microorganism under active investigation. Laboratory preparations or infectious agents usually have titers grossly in excess of those normally observed in nature. The decontamination of these high-titer materials presents certain problems. Maintenance systems for bacteria or viruses are specifically selected to preserve viability of the agent. Agar, proteinaceous nutrients, and cellular materials can be extremely effective in physically retarding or chemically binding active moieties of chemical decontaminants. Such interferences with the desired action of decontaminants may require the use of decontaminant concentrations and contact times in excess of those shown to be effective in the test tube. Similarly, a major portion of decontaminant contact time required to achieve a given level of agent inactivation may be expended in inactivating a relatively small number of the more resistant members of the population. The current state of the art provides little information on which to predict the probable virulence of these survivors. These problems are, however, common to all potentially pathogenic agents and must always be considered in selecting decontaminants and procedures for their use.

Microorganisms exhibit a range of resistance to chemical decontaminants. In terms of practical decontamination, most vegetative bacteria, fungi and lipid-containing viruses, are relatively susceptible to chemical decontamination. The non-lipid-containing viruses and bacteria with a waxy coating such as tubercle bacillus occupy a mid-range of resistance. Spore forms are the most resistant.

A decontaminant selected on the basis of its effectiveness against microorganisms on any range of the resistance scale will be effective against microorganisms lower on the scale. Therefore, if decontaminants that effectively control spore forms are selected for routine laboratory decontamination, it can be assumed that any other microorganisms generated by laboratory operations, even in high concentrations, would also be inactivated.

An additional area that must be considered and for which there is little definitive information available is the "inactivation" of nucleic acids. Nucleic acids often have better survival characteristics under adverse conditions than do the intact virions and cells from which they were derived. Strong oxidizers, strong acids and bases, and either gaseous or aqueous formaldehyde should react readily with nucleic acids. Their ability to destroy the nucleic acid being studied, however, should be confirmed in the experimenter's laboratory. Because of innate differences in the chemistry of RNA and DNA the effectiveness of a decontaminant for one cannot be extrapolated to the other. For example, RNA molecules

are susceptible to mild alkaline hydrolysis by virtue of the free hydroxyl group in the 2' position, whereas DNA molecules are not susceptible to mild alkaline hydrolysis.

Table II summarizes pertinent characteristics and potential applications for several categories of chemical decontaminants most likely to be used in the biological laboratory. Practical concentrations and contact times that may differ markedly from the recommendations of manufacturers of proprietary products are suggested. It has been assumed that microorganisms will be afforded a high degree of potential protection by organic menstruums. It has not been assumed that a sterile state will result from application of the indicated concentrations and contact times. It should be emphasized that these data are only indicative of efficacy under artificial test conditions. The efficacy of any of the decontaminants should be conclusively determined by individual investigators. It is readily evident that each of the decontaminants has a range of advantages and disadvantages as well as a range of potential for inactivation of a diverse microflora. Equally evident is the need for compromise as an alternative to maintaining a veritable "drug store" of decontaminants.

APPENDIX D

TABLE II

SUMMARY OF PRACTICAL DECONTAMINANTS FOR USE IN THE LABORATORY

| DECONTAMINANTS | | PRACTICAL REQUIREMENTS | | | | | INACTIVATES | | | | IMPORTANT CHARACTERISTICS | | | | | | | | | | | | POTENTIAL APPLICATION | | | | | | | | | | PROPRIETARY DECONTAMINANTS EXAMPLES [6] |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | CONCENTRATION ACTIVE INGREDIENT | CONTACT TIME (Minutes) | | | | | | | | EFFECTIVE SHELF LIFE > 1 WEEK [4] | CORROSIVE | FLAMMABLE | EXPLOSION POTENTIAL | RESIDUE | INACTIVATED BY ORGANIC MATTER | LENS COMPATIBLE [7] | ELECTRONICS COMPATIBLE | SKIN IRRITANT | EYE IRRITANT | RESPIRATORY IRRITANT | TOXIC [5] | WORK SURFACES | DIRTY GLASSWARE | LARGE AREA DECON. | AIR HANDLING SYSTEMS | PORTABLE EQUIP. SURFACE DECON. | PORTABLE EQUIP. PENETRATING DECON. | STATIONARY EQUIP. SURFACE DECON. | STATIONARY EQUIP. PENETRATING DECON. | LENSES & ELECTRONIC INSTRUMENTS | LIQUIDS FOR DISCARD | BOOKS, PAPERS | |
| TYPE | CATEGORY | | LIPO VIRUS ONLY | BROAD SPECTRUM | TEMP. °C | REL. HUMID. % | VEG. BACT. | LIPO VIRUS | HYDROPHILIC VIRUS | BACT. SPORES | | | | | | | | | | | | | | | | | | | | | | | |
| LIQUID | Quat. Ammon. CPDS | 2% | 10 | N.E. | | | + | + | | | + | | | | | + | + | | + | + | | + | ✓ | ✓ | | | ✓ | | ✓ | | | | A-33, CDQ, END-BAC, HI-TOR, MIKRO-QUAT |
| | Phenolic CPDS | 2% | 10 | N.E. | | | + | + | 1 | | + | + | | | + | | | | + | + | | + | ✓ | ✓ | | | ✓ | | ✓ | | | | HIL-PHENE, MATAR, MIKRO-BAC, O-SYL |
| | Chlorine CPDS | 3% | 10 | 30 | | | + | + | + | + | | + | | | + | + | | | + | + | + | + | ✓ | ✓ | | | ✓ | | ✓ | | | ✓ | CHLORAMINE T, CLOROX, PUREX |
| | Iodophor | 2% | 10 | 30 | | | + | + | + | + | + | + | | | + | + | | | + | + | | + | ✓ | ✓ | | | ✓ | | ✓ | | | . | HI-SINE, IOPREP, MIKROXLENE, WESCODYNE |
| | Alcohol, Ethyl | 85% | 10 | N.E. | | | + | + | 1 | | + | | + | | | | | | | + | | + | ✓ | ✓ | | | ✓ | | ✓ | | | | |
| | Alcohol, Isopropyl | 85% | 10 | N.E. | | | + | + | 1 | | + | | + | | | | | | | + | | + | ✓ | ✓ | | | ✓ | | ✓ | | | | |
| | Formaldehyde | 8% | 10 | 30 | | | + | + | + | + | + | | | | + | | | | + | + | | + | ✓ | ✓ | | | ✓ | | ✓ | | | | STERAC |
| | Glutaraldehyde | 2% | 10 | 30 | | | + | + | + | + | + | | | | + | | + | | + | + | | + | ✓ | ✓ | | | ✓ | | ✓ | | | | CIDEX |
| GAS | Ethylene Oxide | .45g/l | 60 | 60 | 37 | 30 | + | + | + | + | N.A. | | + [2] | + [2] | | | + | + | + | + | + | + | | | | | | ✓ | | | ✓ | | ✓ | CARBOXIDE, CRYOXCIDE, STEROXCIDE |
| | Paraformaldehyde | .3g/ft [3] | 60 | 60 | > 23 | > 60 | + | + | + | + | N.A. | | + [3] | + [3] | | | + | + | + | + | + | + | | | ✓ | ✓ | | ✓ | | ✓ | ✓ | | | |

N.A. = Not applicable - N.E. = Not effective

1. Variable results dependent on virus.
2. Neither flammable or explosive in 90% $CO_2$ or fluorinate hydrocarbon use form.
3. At concentrations of 7 to 73% by volume in air, solid-exposure to open flame.
4. Protected from light and air.
5. By skin or mouth or both - refer to manufacturer's literature and/or Merck Index.
6. Space limitations preclude listing all products available. Individual listings (or omissions) do not imply endorsement or rejection of any product by the NIH.
7. Refers to microscope and camera lenses.

## VII. Housekeeping

### A. Introduction

Well-defined housekeeping procedures and schedules are essential in reducing the risks of working with etiologic agents and in protecting the integrity of the research program. This is particularly true in the biological laboratory operating under less than total containment concepts and in all areas used for the housing of animals, whether or not they have been intentionally infected. A well-conceived and well-executed housekeeping program limits physical clutter that could distract the attention and interfere with the activities of laboratory personnel at a critical moment in a potentially hazardous procedure, provides a work area that will not in itself be a source of physical injury or contamination, and provides an area that promotes the efficient use of decontaminants in the event of the inadvertent release of a harmful agent. Less immediately evident are the benefits of establishing, among personnel of widely varying levels of education, an appreciation of the nature and sources of biological contamination.

Housekeeping is an omnibus term that can be interpreted as broadly or as narrowly as one chooses. It can be seen that many of the procedures found under special headings, such as decontamination, disposal, and animal care, are, in reality, specific instructions for safely accomplishing otherwise routine housekeeping chores. In these safety suggestions for research on recombinant DNA molecules, it has been elected to address specifically only tasks of a janitorial nature under the subject of housekeeping.

The objectives of housekeeping in the biological laboratory are to:

1. Provide an orderly work area conducive to the accomplishment of the research program.
2. Provide work areas devoid of physical hazards.
3. Provide a clean work area with background contamination ideally held to a zero level but more realistically to a level such that extraordinary measures in sterile techniques are not required to maintain integrity of the biological systems being researched.
4. Prevent the accumulation of materials from current and past experiments that constitute a hazard to laboratory personnel.
5. Prevent the creation of aerosols of hazardous materials as a result of the housekeeping procedures used.

Procedures developed in the area of housekeeping should be based on the highest level of risk to which the personnel and integrity of the experiments will be subject. Such an approach avoids the confusion of multiple practices and retraining of personnel. The primary function, then, of routine housekeeping procedures is to prevent the accumulation of organic debris that (i) may harbor microorganisms that are a potential threat to the integrity of the biological systems under investigation, (ii) may enhance the survival of microorganisms inadvertently released in experimental procedures, (iii) may retard penetration of decontaminants, (iv) may be transferable from one area to another on clothing and shoes, (v) may, with sufficient buildup, become a

biohazard as a consequence of secondary aerosolization by personnel and air movement, and (vi) may cause allergenic sensitization of personnel, e.g., to animal danders.

Housekeeping in animal care units has the same primary function as that stated for the laboratory and should, in addition, be as meticulously carried out in quarantine and conditioning areas as in areas used to house experimentally infected animals. No other areas in the laboratory have the constant potential for creation of significant quantities of contaminated organic debris than do animal care facilities.

In all laboratories, efforts to achieve total decontamination and to conduct a major cleanup of the biological complex are normally undertaken at relatively long time intervals. Routine housekeeping must be relied on to provide a work area free of significant sources of background contamination. The provision of such a work area is not simply a matter of indicating in a general way what has to be done, who will do it, and how often. The supervisor must view each task critically in terms of the potential biohazard involved, decide on a detailed procedure for its accomplishment, and provide instructions to laboratory personnel in a manner that minimizes the opportunity for misunderstanding.

The following checklist outlines a portion of the items requiring critical review by the laboratory supervisor. It is not intended to be complete but is presented as an example of the detailed manner in which housekeeping in the biological laboratory complex must be viewed.

Administration Areas
Aisles
Animal Food Storage

Animal Bedding Storage
Biological Safety Cabinets
Bench Tops and Other Work Surfaces
Ceilings
Change Rooms
Cleaning Solution Disposal
Cages and Cage Racks
Dry Ice Chests
Deep Freeze Chests
Entry and Exit Ways
Equipment Storage
Floors
Glassware
General Laboratory Equipment Cleanup
Hallways
Incubators
Instruments
Insect and Rodent Control
Light Fixtures
Mechanical Equipment Areas
Mops
Pipes - Wall and Ceiling Hung
Refrigerators
Showers
Supply Storage
UV Lamps
Vacuum Cleaners
Waste Accumulations
Waste Water Disposal
Others

Housekeeping in the laboratory is one of the avenues that leads to accomplishing the research program safely. It is important that housekeeping tasks be assigned to personnel who are knowledgeable of the research program and special hazards of the research environment. The recommended approach to housekeeping is the assignment of housekeeping tasks to the research teams on an individual basis for their immediate work areas and on a cooperative basis for areas of common usage. Similarly, animal caretaker personnel should be responsible for housekeeping

in animal care areas. The laboratory supervisor must determine the frequency with which the individual and cooperative housekeeping chores need be accomplished. He should provide schedules and perform frequent inspection to assure compliance. This approach assures that research work flow patterns will not be interrupted by an alien cleanup crew, delicate laboratory equipment will be handled only by those most knowledgeable of its particular requirements, and the location of concentrated biological preparations and contaminated equipment used in their preparation and application will be known.

B. Floor Care

Avoidance of dry sweeping and dusting will reduce the formation of nonspecific environmental aerosols. Wet mopping or vacuum cleaning with a high-efficiency particulate air (HEPA) filter on the exhaust is recommended.

Careful consideration must be given to design and quality in the selection of cleaning equipment and materials and in their use to prevent the substitution of one hazard for another.

In the absence of overt hazardous spills, the cleaning process commonly will consist of an initial vacuuming to remove all gross particulate matter and a follow-up wet mopping with a solution of chemical decontaminant containing a detergent. Depending on the nature of the surfaces to be cleaned and availability of floor drains, removal of residual cleaning solutions can be accomplished by a number of methods.

Among these are: pickup with a partially dry mop, pickup with a wet vacuum that has an adequately filtered exhaust, or removal to a convenient floor drain by use of a floor squeegee.

After cleaning up a spill of infected material, the residual solution should not be discharged to a sanitary sewer until it has been autoclaved or given further chemical treatment, such as by the addition of sodium hypochlorite sufficient to provide a final concentration of 500 ppm chlorine. Most household bleaches are marketed with a chlorine content of 5.25%. These in a final dilution of 1:100, yield 525 ppm of available chlorine. After allowing a contact time of 15 minutes, these solutions may be flushed down any available drain. Chlorine solutions in these high concentrations may be too corrosive for general application to floors and equipment. In any event, if solutions are used in this way, after the contact time the area should be rinsed with water.

C. Dry Sweeping

While it is recommended that dry sweeping be minimized, this may be the only method available or practicable under certain circumstances. In such cases, sweeping compounds used with push brooms and dry-dust mop heads treated to suppress aerosolization of dust should be used.

Sweeping compounds available from the usual janitorial supply firms fall in three categories:

- wax-based compounds used on vinyl floors and waxed floor coverings.
- oil-based compounds for concrete floors.

- oil-based compounds with abrasives (such as sand) to achieve a dry scouring action where much soil is present.

Dry-dust mop heads can be purchased as treated disposable units or as reusable, washable heads that must be treated with appropriate sprays or by other means to improve their dust-capturing property.

D. Vacuum Cleaning

In the absence of a HEPA filter on the exhaust, the usual wet and dry industrial-type vacuum cleaner is a potent aerosol generator. The HEPA-filtered exhaust used in conjunction with a well-sealed vacuum unit, however, can negate this factor because of its ability to pass large volumes of exhaust air while retaining particles with a minimum efficiency of 99.97%. Wet and dry units incorporating a HEPA filter on the exhaust are available from a number of manufacturers.

There are no particular requirements with respect to the manner in which the dry vacuuming is accomplished other than to emphasize that the objective is to remove all debris and particulate matter. The manufacturer's directions adequately detail the frequency of bag changes, filter changes, and mechanical adjustments.

Dry material vacuum-collected during these floor-cleaning activities is potentially contaminated, but the nature of the risk is probably greater to the experiment than to the experimenter. It is wise to effect bag and filter changes and to clean out collection tanks in a manner that will avoid or minimize aerosolizing the contents of the vacuum cleaner.

A vacuum machine that collects debris in a disposable bag is preferable to machines that collect the major debris in a tank and on an exposed primary filter. Even though it may serve as a primary filter, the disposable bag must be removed with caution. A bellows effect may pump dust out of the bag if its intake opening is not sealed before moving it to a plastic bag for transfer out of the area. In any event, the outer surface of the disposable bag will probably bear some dust contamination, which also may occur on inner surfaces of the machine.

To avoid contaminating experimental materials, the emptying of vacuum collection tanks and changing of bags and filters are best done away from the immediate laboratory area, for example, in a small area that can be easily cleaned afterwards. The use of heavy rubber gloves is recommended when removing wastes from tanks in case broken glass is present. After making the filter changes, all external surfaces of the immediate work area and the equipment should be wiped with a cloth moistened in decontaminant. The operator might plan for a change of laboratory clothing afterwards so as to minimize carrying contamination into other areas of the laboratory.

Avoid use of dry vacuum cleaning equipment in work with high risk agents in the open laboratory. Should it be necessary to use it, it is recommended that gaseous sterilization be used to minimize aerosolization of microorganisms before waste is emptied from the vacuum container. Because complete penetration of sterilizing gases into the collected dry dust may be a problem, all wastes should be placed in a plastic bag, which then is tightly closed and incinerated or disposed of in an approved manner.

When dry vacuum cleaning equipment has been used within a gastight safety cabinet system, it can be treated in an attached double-door carboxyclave (an autoclave equipped with an ethylene oxide gas sterilization system) to allow for removal and emptying of the collection tank.

If a wet vacuum is to be used for pickup of the detergent-germicide solution from the floor, the manufacturer's recommendations on filter life should be followed. In addition, the operation of the vacuum should be closely observed for evidence of operating changes indicating restricted airflow or, conversely, increased flow indicating filter failure. Liquids collected in the vacuum cleaner after floor mopping will contain decontaminant material. These liquids may be poured down a convenient floor drain, except in the case of cleanup wastes from an overt spill. The collected liquid should then be autoclaved or treated with chlorine solution before disposal.

Provisions should be made for regular decontamination of the entire vacuum cleaner with formaldehyde gas or vapor, or ethylene oxide. This should be done after use if the vacuum is used in any manner for cleanup of overt spills of infectious material.

E. Selection of a Cleaning Solution

The selection of a detergent-decontaminant combination for routine cleaning of the laboratory complex should be based on the requirements of the area of greatest potential for contamination by the widest spectrum of microorganisms. With rare exception, this will be identified as the animal holding area and the expected microorganisms may well include fungi,

viruses, and the vegetative and spore forms of bacteria. A decontaminating solution for such a range of microorganisms would, however, be expensive and excessively corrosive for routine use. Except in those rare instances where it can be assumed that pathogenic spores are being shed by laboratory animals, the risks from the spores are more likely to affect the experiments than the personnel. The spores tend to be associated with organic debris from bedding and food, thus offering potential for removal or at least a large initial reduction in their numbers by vacuum cleaning. A wide range of cleaning solutions that are mildly sporicidal, reasonably residual, and are not destructive to the physical plant are available. Phenol derivatives in combination with a detergent have these characteristics and have been selected for routine use in a number of research facilities. There are numerous detergent-phenolic combinations available on the market. The phenols are one type of a broad spectrum of biocidal substances that include the mercurials, quaternary ammonium compounds, chlorine compounds, iodophores, alcohols, formaldehyde, glutaraldehyde, and combinations of alcohol with either iodine or formaldehyde. These have been discussed in Section VI.

The laboratory supervisor should make a selection from those types most readily available which meet the general criteria of effectiveness, residual properties, and low corrosiveness.

F. Wet Mopping - Two-Bucket Method

Wet mopping of floors in laboratory and animal care areas is, from a safety standpoint, most conveniently and efficiently accomplished using a two-bucket system. The principal feature of such a system is that fresh

detergent-decontaminant solution is always applied to the floor from one bucket, while all spent cleaning solution wrung from the mop is collected in the second bucket. Compact dolly-mounted double-bucket units with foot-operated wringers are available from most janitorial supply houses. A freshly laundered mop head of the cotton string type should be used daily. This requires that a mop with removable head be provided as opposed to a fixed-head type. In practice, the mop is saturated with fresh solution, very lightly wrung into the second bucket and applied to the floor using a figure eight motion of the mop head. After every four or five strokes, the mop head is turned over and the process continued until an area of approximately 100 $ft^2$ has been covered. After allowing a contact time of five minutes, the solution is removed with either a wet vacuum cleaner with HEPA-filtered exhaust or with the wrung-out mop. The mopping is continued in 100 $ft^2$ increments until the total floor area has been covered. Floor-cleaning procedures are most effectively completed after the majority of the work force has departed and should progress from areas of least potential contamination to those of greatest potential. Before a mop head is sent to a laundry, it should be autoclaved. Spent cleaning fluids are disposed of by flushing down the drain.

If the cleanup follows an overt spill of infectious material, the spent cleaning solution, after removal from the floor, should be autoclaved or treated with chlorine colution. Chlorine (as household bleach) should be added to give 500 ppm and held for a contact time of 15 minutes before dumping in the sanitary sewer.

G. Alternative Floor Cleaning Method for Animal Care Areas and Areas with Monolithic Floors

The absence of permanently placed laboratory benches and fixed equipment, coupled with the mobility of modern cage racks, makes possible alternate floor-cleaning procedures in animal care facilities. As in all considerations of methodologies in biomedical laboratory facilities, it is necessary to assess the compatibility of procedures and facilities from the hazard point of view. The alternative floor-cleaning procedure to be discussed requires that floors are completely sealed or of monolithic construction so that liquid leakage to adjacent areas does not occur and that floor drains or wet vacuum cleaners are available.

Subsequent to the removal of all debris by dry vacuum, move the cage racks to one side of the room. Cover the floor of the remaining cleared portion of the room with detergent-decontaminant solution applied at a rate of approximately one gallon per 144 $ft^2$ from a one-gallon tank sprayer, using a setting of the nozzle which will cause the solution to flow on and not create a spray. The nozzle is placed close to the floor. Allow a fifteen-minute contact period; then push the cleaning solution to the floor drain with a large floor squeegee or pick it up with a wet vacuum. Allow the floor to air dry; move the cage racks into the cleaned area, and repeat the process for the remaining floor area. Floor drains in these areas should be rim-flush, at least six inches in diameter, and fitted with a screen or porous trap bucket to catch large debris that escapes the initial dry cleaning. Such screens and baskets should be emptied after treatment with a decontaminant. If space utilization does not require frequent floor washdown, pour a half-gallon of detergent-decontaminant solution into the drain each week to keep the trap in the waste line filled against backup of sewer gases.

VIII. Clean-Up of Biohazardous Spills (8,9,10)

A. Biohazardous Spill in a Biological Safety Cabinet

Chemical decontamination procedures should be initiated at once while the cabinet continues to operate to prevent escape of contaminants from the cabinet.

1. Spray or wipe walls, work surfaces, and equipment with a 2% solution of an iodophor-decontaminant (Wescodyne or equivalent). A decontaminant detergent has the advantage of detergent activity, which is important because extraneous organic substances frequently interfere with the reaction between the microorganisms and the active agent of the decontaminant. Operator should wear gloves during this procedure.
2. Flood the top work surface tray, and, if a Class II cabinet, the drain pans and catch basins below the work surface, with a decontaminant and allow to stand 10-15 minutes.
3. Remove excess decontaminant from the tray by wiping with a sponge or cloth soaked in a decontaminant. For Class II cabinents, drain the tray into the cabinet base, lift out tray and removable exhaust grille work, and wipe off top and bottom (underside) surfaces with a sponge or cloth soaked in a decontaminant. Then replace in position and drain decontaminant from cabinet base into appropriate container and autoclave according to standard procedures. Gloves, cloth or sponge should be discarded in an autoclave pan and autoclaved.

B. Biohazard Spill Outside a Biological Safety Cabinet

1. Hold your breath, leave the room immediately, and close the door.
2. Warn others not to enter the contaminated area.
3. Remove and put into a container contaminated garments for autoclaving and thoroughly wash hands and face.
4. Wait 30 minutes to allow dissipation of aerosols created by the spill.
5. Put on a long-sleeve gown, mask, and rubber gloves before reentering the room. (For a high risk agent, a jumpsuit with tight-fitting wrists and use of a respirator should be considered).
6. Pour a decontaminant solution (5% iodophor or 5% hypochlorite are recommended) around the spill and allow to flow into the spill. Paper towels soaked with the decontaminant may be used to cover the area. To minimize aerosolization, avoid pouring the decontaminant solution directly onto the spill.
7. Let stand 20 minutes to allow an adequate contact time.
8. Using an autoclavable dust pan and squeegee, transfer all contaminated materials (paper towels, glass, liquid, gloves, etc.) into a deep autoclave pan. Cover the pan with aluminum foil or other suitable cover and autoclave according to standard directions.

9. The dust pan and squeegee should be placed in an autoclavable bag and autoclaved according to standard directions. Contact of reusable items with non autoclavable plastic bags should be avoided --- separation of the plastic after autoclaving can be very difficult.

C. Radioactive Biohazard Spill Outside a Biological Safety Cabinet

In the event that a biohazardous spill also involves a radiation hazard, the clean-up procedure may have to be modified, depending on an evaluation of the risk assessment of relative biological and radiological hazard.

Laboratories handling radioactive substances must have the services of a designated radiation protection officer available for consultation.

The following procedure indicates suggested variations from the biohazard spill procedure (above) that should be considered when a radioactive biohazard spill occurs outside a Biological Safety Cabinet.*

1. Holding your breath, leave the room immediately and close the door.
2. Warn others not to enter the contaminated area.
3. Remove and put in a container contaminated garments for autoclaving and thoroughly wash hands and face.
4. Wait thirty minutes to allow dissipation of aerosols created by the spill.

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*Changes in procedures have been starred and underlined.

*Before clean-up procedures begin, a radiation protection officer should survey the spill for external radiation hazard to determine the relative degree of risk.

5. Put on a long-sleeve gown, mask, and rubber gloves before reentering the room. (For a high risk agent, a jumpsuit with tight-fitting sleeves and a respirator should be considered).
6. Pour a decontaminant solution (5% iodophor or 5% hypochlorite are recommended) around the spill and allow to flow into the spill. Paper towels soaked with the decontaminant may be used to cover the area. To minimize aerosolization, avoid pouring the decontaminant solution directly onto the spill.
7. Let stand 20 minutes to allow adequate disinfectant contact time.
8. *In most cases, the spill will involve $^{14}C$ or $^{3}H$, which present no external hazard. However, if more energetic beta or gamma emitters are involved, care must be taken to prevent hand and body radiation exposure. The radiation protection officer must make this determination before the clean-up operation is begun.

If the radiation protection officer approves, the biohazard-handling procedure may begin: Using an autoclavable dust pan and squeegee, transfer all contaminated materials (paper towels, glass, liquid, gloves, etc.) into a deep autoclave pan. Cover the pan with aluminum foil or other suitable cover and autoclave according to standard directions.

*If the radiation protection officer determines that radioactive vapors may be released and thereby contaminate the autoclave, the material must not be autoclaved. In that case, sufficient decontaminant solution to immerse the contents should be added to the waste container. The cover should be sealed with waterproof tape, and the container stored and handled for disposal as radioactive waste. Radioactive and biohazard warning symbols should be affixed to the waste container. As a general rule, autoclaving should be avoided.

9. If autoclaving has been approved, the dust pan and squeegee should be placed in an autoclavable bag and autoclaved according to standard directions. Contact of reusable items with plastic bags should be avoided -- separation of the plastic after autoclaving can be very difficult.

*A final radioactive survey should be made of the spill area, dust pan, and squeegee with a Geiger counter, or a smear should be taken and counted in a liquid scintillation counter.

## IX. A Secondary Reservoir and Filtration Apparatus for Vacuum Systems

The aspiration of tissue culture media from monolayer cultures and of supernatants from centrifuged samples into collection vessels or reservoirs is a common procedure in many laboratories. To prevent the accidental contamination by aerosols or fluids of house vacuum systems or laboratory pumps, some investigators have installed side arm flasks containing cotton, sulfuric acid or decontaminant between the reservoir and the vacuum line. Cotton is not completely effective as a filtering agent, sulfuric acid will corrode pipes, and dcontaminants may lose their inactivating ability upon standing. The introduction of a cartridge-type filter that is moisture resistant and has a rated capacity to remove particles 350 nm (0.35u) or larger in size provides an effective barrier to virus aerosols.

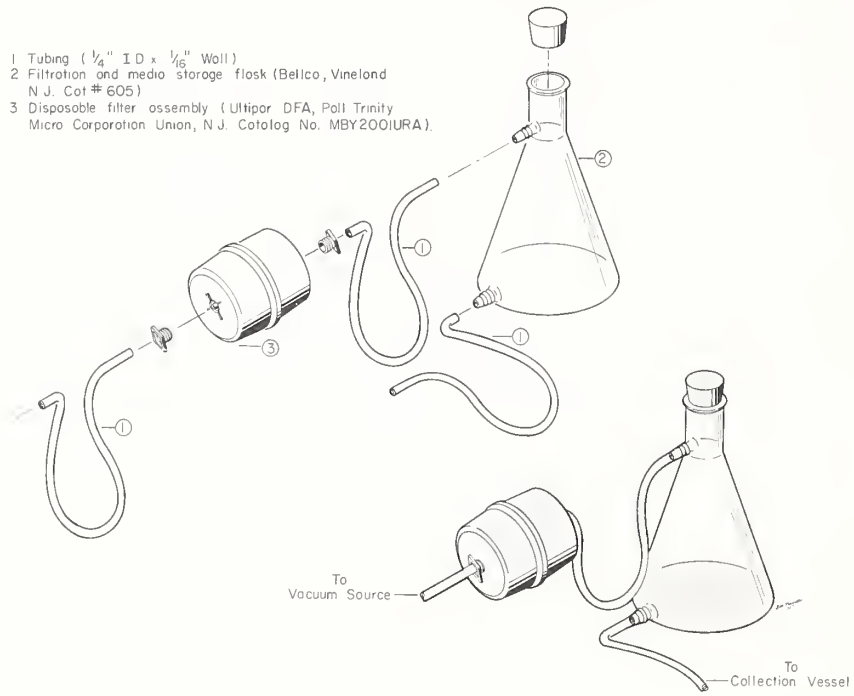
The secondary reservoir and filtration apparatus can be assembled from readily available units as shown in Figure 1. A length of plastic tubing 1/4 inch I.D. x 1/16 inch wall is attached at one end of the reservoir and at the other end to the lower arm of a filtration and media storage flask. These flasks vary in capacity from 250 to 4000 ml, the choice of flask depending on available space and amount of fluid that could be accidentally aspirated. A second tube of the same dimensions is attached from the upper arm of the flask to the inlet port of the disposable filter assembly. The third tube is attached from the filter assembly to a vacuum source. The tubes are securely held to the filter by fittings supplied with the filter and the other tubing connections can be secured by worm drive hose clamps.

Ideally the flask should be placed higher than the reservoir of collection vessel. If fluid is accidentally drawn into the flask, the liquid can drain back into the reservoir by gravity if the connection at the vacuum line is broken. This prevents the loss of fluid which the investigator needs to retain.

Should the flask be used only for the recovery and storage of waste fluids, then the addition of a few grams of Dow Corning Antifoam A to the flask will reduce violent foaming of fluids aspirated into it. Such fluids can be decontaminated by introducing into the reservoir a final 5% concentration of an iodophor or other appropriate decontaminant, holding for 30 minutes and draining as above.

If the filter becomes contaminated or requires changing, the filter and flask can be safely removed by clamping the line between filter and vacuum source. The filter and flask should be autoclaved before the filter is discarded. A new filter can then be installed and the assembly replaced.

Figure 1
A SECONDARY RESERVOIR AND FILTRATION APPARATUS



## X. Packaging and Shipping

### A. Introduction

Federal regulations and carrier tariffs have been promulgated to ensure the safe transport of hazardous biological materials. The NIH Guidelines specify that all DNA recombinant materials will be packaged and shipped in containers that meet the requirements of these regulations and carrier tariffs. In addition when any portion of the recombinant DNA material is derived from an etiologic agent listed in paragraph (c) of 42 CFR 72.25 (which is included at the end of this section, page D-85) the labeling requirements in these regulations and carrier tariffs shall apply.

### B. Packaging of Recombinant DNA Materials

1. Volume less than 50 ml.

Material shall be placed in a securely closed, watertight container [primary container (test tube, vial, etc.)] which shall be enclosed in a second, durable watertight container (secondary container). Several primary containers may be enclosed in a single secondary container, if the total volume of all the primary containers so enclosed does not exceed 50 ml. The space at the top, bottom, and sides between the

primary and secondary containers shall contain sufficient non-particulate absorbent material to absorb the entire contents of the primary container(s) in case of breakage or leakage. Each set of primary and secondary containers shall then be enclosed in an outer shipping container constructed of corrugated fiberboard, cardboard, wood, or other material of equivalent strength.

If dry ice is used as a refrigerant, it must be placed outside the secondary container(s).

Descriptions of this packaging method are given in Table III.

2. Volumes of 50 ml or Greater.

Material shall be placed in a securely closed, watertight container (primary container) which shall be enclosed in a second, durable watertight container (secondary container). Single primary containers shall not contain more than 500 ml. of material. However, two or more primary containers whose combined volumes do not exceed 500 ml. may be placed in a single secondary container. The space at the top, bottom, and sides between the primary and secondary containers shall contain sufficient non-particulate absorbent material to absorb the entire contents of the primary container(s) in case of breakage or leakage. Each set of primary and secondary containers shall then be enclosed in an outer shipping container constructed of corrugated fiberboard, cardboard, wood, or other

material of equivalent strength. A shock absorbent material, in volume at least equal to that of the absorbent material between the primary and secondary containers, shall be placed at the top, bottom, and sides between the secondary container and the outer shipping container. Not more than eight secondary shipping containers may be enclosed in a single outer shipping container. (The maximum amount of materials which may be enclosed within a single outer shipping container should not exceed 4,000 ml.).

If dry ice is used as a refrigerant, it must be placed outside the secondary container(s). If dry ice is used between the secondary container and the outer shipping container, the shock absorbent material shall be placed so that the secondary container does not become loose inside the outer shipping container as the dry ice sublimates.

Descriptions of packages which comply with the regulations of the Department of Transportation (DOT) are given in Table IV.

C. Labeling of Packages Containing Recombinant DNA Materials

1. Materials which do not contain any portion of an etiologic agent listed in paragraph (c) of 42 CFR 72.25.

Material data forms, letters, and other information identifying or describing the material should be placed around the outside of the secondary container. Place only the address label on the outer shipping container.

DO NOT USE THE LABEL FOR ETIOLOGIC AGENTS/BIOMEDICAL MATERIAL.

2. Materials which contain any portion of an etiologic agent listed in paragraph (c) of 42 CFR 72.25.

   Material data forms, letters, and other information identifying or describing the material should be placed around the outside of the secondary container. In addition to the address label, the label for Etiologic Agents/Biomedical Material must be affixed to the outer shipping container. This label is described in paragraph (c) (4) of 42 CFR 72.25.

3. Materials which contain any portion of a plant pest (plant pathogens) which are so defined by the Department of Agriculture (USDA).

   Material data forms, letters, and other information identifying or describing the material should be placed around the outside of the secondary container. In addition to the address label, the shipping labels furnished by the USDA as part of the General, Courtesy, or Special Permits required for research with and shipment of such agents shall be affixed to the outer shipping container.

D. Additional Shipping Requirements and Limitations for Recombinant DNA Materials.

1. Domestic Transportation.

Civil Aeronautics Board Rule No. 82 (Air Transport Association Restricted Articles Tariff 6-D) requires that a Shipper's Certificate, depicted below, be completed and affixed to all shipments which bear the ETIOLOGIC AGENT/BIOMEDICAL MATERIALS label required under the provisions of the Interstate Quarantine regulations [42 CFR Section 72.25(c)]. The Certificate must be completed in duplicate and affixed to the outer shipping container.

This is to certify that the contents of this consignment are properly classified, described by proper shipping name and are packed, marked and labelled and are in proper condition for carriage by air according to all applicable carrier and government regulations. (For international shipments add "and to the IATA Restricted Articles Regulations".) This consignment is within the limitations prescribed for: PASSENGER AIRCRAFT/CARGO ONLY (cross out nonapplicable).

| Number of Packages | Specify Each Article Separately (Proper Shipping Name) | Classification | Net Quantity per Package |
|---|---|---|---|
| | ETIOLOGIC AGENT, n.o.s. | ETIO. AG. | |

Shipper:

Date _____

_____

(Signature of Shipper)

Shipments of recombinant DNA Materials exceeding 50 ml in volume and containing any portion of an etiologic agent listed in paragraph (c) of 42 CFR 72.25 are restricted, by DOT regulations, to transport by cargo only aircraft. When the volume

of a single primary container exceeds the 50 ml limitation, this restriction must be indicated on the Shipper's Certificate by crossing out "Passenger Aircraft".

When dry ice is used as a refrigerant an "ORA -Group A-DRY ICE LABEL" should be affixed to the outer shipping container. The amount of dry ice used and the date packed should be designated on the label.

2. International Transportation

In addition to the packaging and labeling requirements of the regulations previously cited, international shipments of recombinant DNA materials in which any portion of the material is derived from an etiologic agent listed in paragraph (c) of 42 CFR 72.25 must have one or more of the following documents — depending on the country of destination:

(1) Parcel Post Customs Declaration (PS 2966) tag.

(2) Parcel Post Customs Declaration (PS 2966-A) label.

(3) International Parcel Post - Instructions Given by Sender (POD 2922) label.

(4) Dispatch note (POD 2972) tag.

(5) "Violet Label"

(6) Shipper's Certificate specified in the current International Air Transport Association Tariff.

Individual country requirements are listed in "International Postage Rates and Fees" (USPO Publication 51).

TABLE III

Description of Packages for Material in Volumes less than 50 ml.

| Volume (ml) | Primary Container | Packing | Secondary Container | Packing | Outer Shipping d/ Container |
|---|---|---|---|---|---|
| 15 max. | Sealed vial(s) or small glass test tube, screw cap* or stopper, taped | a/ | Metal can 1" diam. x 7" O.D. metal screw cap | None Required | Fiberbody; metal screw cap, top and bottom; 1-1/2" diam. x 7 to 7-1/2" O.D. |
| 50 or less | One 20 x 150 mm test tube, taped* stopper or multiple small vials | a/ | Metal can 2-1/2" diam. x 6-1/2" high O.D. screw cap | None Required | Fiberbody; metal screw cap, top and bottom; 3-1/4" diam. x 7 to 7-1/2" O.D. |
| 50 or less | Plastic* screw cap* bottle or Pyrex glass with skirt rubber stopper | a/ | Metal can 2-1/2" diam. x 6-1/2" high O.D. screw cap | None Required | Fiberbody; metal screw cap, top and bottom; 3-1/4" diam. x 7 to 7-1/2" O.D. |
| 50 or less | Multiple watertight vials or * tubes, taped stoppers | a/ | One or more friction-seal tin cans b/ 306 x 400 or larger | c/ | Fiberboard box |

*The flexibility of the plastic bottle requires that a stopper or screw cap be secured in place by adhesive tape. The usual equivalent-size glass flat-sided prescription bottle is too fragile for use. For air transport, all stoppers, corks, and caps on primary containers must be secured in place with wire, tape, or other means, and all screw-capped containers of unfrozen liquid must be placed in 5 or 6 mil polyvinyl tubing heat-sealed at both ends to prevent atmospheric decompression that may result in leakage past the screw cap.

O.D. = outside dimensions.

a/ Nonparticulate absorbent material at top, bottom and sides that will completely absorb contents of the primary container(s).

b/ 610 x 708 and 804 x 908 are trade designations for outside dimensions of 6-10/16 inches diameter x 7-8/16" height, and 8-4/16" x 9-8/16".

c/ None required, but with the 306 x 400 cans or larger cans use sufficient nonparticulate shock-absorbent material to prevent rattling.

d/ If materials are to be refrigerated, it is recommended that an overpack be used to contain the refrigerant and the secured (original) outer shipping container. A leak proof outer container must be used for water ice. If dry ice is used the outer container must permit release of carbon dioxide. Interior supports must be provided to hold the container(s) in the original position(s) after wet or dry ice has dissipated.

TABLE IV

Description of Packages for Material in Volumes of 50 ml or greater

| Volume (ml) | Primary Container | Packing | Secondary Container | Packing: With Refrigerant | Packing: Without Refrigerant | Outer Shipping Container: With Refrigerant | Outer Shipping Container: Without Refrigerant |
|---|---|---|---|---|---|---|---|
| 51 to 100 ml | Plastic* or Pyrex glass screw cap* bottle; rubber or skirt rubber stopper, taped* | a/ | Consists of metal container & outer container specified in Table III | Styrofoam box shock-absorbent insulation | c/ | Fiberboard box closely fitting the styrofoam box, taped shut | Corrugated fiberboard or cardboard box, taped shut |
| 100 max. | One 100 ml plastic* screw cap* narrow neck bottle or Pyrex glass, taped* | a/ | No. 3 crimp seal tin can 404 x 700 or a 1-gallon friction-seal tin can, 610 x 708, top soldered or clipped at 4 points b/ | Styrofoam box shock-absorbent insulation | c/ | Fiberboard box closely fitting the styrofoam box, taped shut | V3C cardboard box PS3 type, 9-3/16" x 9-3/16" x 11-1/4" high O.D. taped shut with 3" type PS3 tape |
| 200 max. | Two 100 ml plastic* screw cap* bottles or Pyrex glass, taped | a/ | No. 3 crimp seal tin can 404 x 700 or a 1-gallon friction-seal tin can, 610 x 708, top soldered or clipped at 4 points b/ | Styrofoam box shock-absorbent insulation | c/ | Fiberboard box closely fitting the styrofoam box, taped shut | V3C cardboard box PS3 type, 9-3/16" x 9-3/16" x 11-1/4" high O.D. taped shut with 3" type PS3 tape |
| 250 max. | One 250 ml, plastic* narrow mouth screw cap* bottle or Pyrex glass skirted rubber stopper, taped* | a/ | No. 3 crimp seal tin can 404 x 700 or a 1-gallon friction-seal tin can, 610 x 708, top soldered or clipped at 4 points b/ | Styrofoam box shock-absorbent insulation | c/ | Fiberboard box closely fitting the styrofoam box, taped shut | V3C cardboard box PS3 type, 9-3/16" x 9-3/16" x 11-1/4" high O.D. taped shut with 3" type PS3 tape |
| 500 max. | Two 250 ml plastic* screw cap* bottles or Pyrex glass bottles, taped* | a/ | 2-gallon friction-seal tin can, 804 x 908, top soldered or clipped at 4 points b/ | Styrofoam box shock-absorbent insulation | c/ | Fiberboard box closely fitting the styrofoam box, taped shut | V3C cardboard box 12-1/4" x 12-1/4" x 10-3/16" high O.D. taped shut with 3" wide PS3 tape. |
| 500 max. | 500 ml Pyrex glass bottle, rubber-skirt stopper, taped, or 500 ml plastic* bottle, narrow or wide mouth, screw cap*, taped | a/ | No. 12 crimp seal tin can 603 x 810 2-gallon friction-seal tin can, 804 x 908, top soldered or clipped at 4 points b/ | Styrofoam box shock-absorbent insulation | c/ | Fiberboard box closely fitting the styrofoam box, taped shut | V3C cardboard box 12-1/4" x 12-1/4" x 10-3/16" high O.D. taped shut with 3" wide PS3 tape. For the No. 12 can a cardboard box is ok taped shut |

*The flexibility of the plastic bottle requires that a stopper or screw cap be secured in place by adhesive tape. The usual equivalent-size glass flat-sided prescription bottle is too fragile for use. For air transport, all stoppers, corks, and caps on primary containers must be secured in place with wire, tape, or other means, and all screw-capped containers of unfrozen liquid must be placed in 5 or 6 mil polyvinyl tubing heat-sealed at both ends to prevent atmospheric decompression that may result in leakage past the screw cap.

O.D. = outside dimensions.

a/ Nonparticulate absorbent material at top, bottom and sides that will completely absorb contents of the primary container(s).

b/ 610 x 708 and 804 x 908 are trade designations for outside dimensions of 6-10/16 inches diameter x 7-8/16" height, and 8-4/16" x 9-8/16".

c/ Shock absorbent material, in volume at least equal to that between the primary and secondary container(s), at the top, bottom, and sides between the secondary container and the outer shipping container. The shock absorbent material shall be so placed that the secondary container(s) does not become loose inside the outer shipping container as the water ice or dry ice is dissipated.

DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE
PUBLIC HEALTH SERVICE
CENTER FOR DISEASE CONTROL
ATLANTA, GEORGIA 30333
Telephone: (404) 633-3311, Ext. 3883

# TITLE 42—PUBLIC HEALTH

## Chapter I—Public Health Service, Department of Health, Education, and Welfare

### SUBCHAPTER F—QUARANTINE, INSPECTION, LICENSING

### PART 72—INTERSTATE QUARANTINE

### Subpart C—Shipment of Certain Things

Section 72.25 of Part 72, Title 42, Code of Federal Regulations, is amended to read as follows:

§ 72.25 Etiologic agents.[1]

(a) *Definitions.* As used in this section:

(1) An "etiologic agent" means a viable microorganism or its toxin which causes, or may cause, human disease.

(2) A "diagnostic specimen" means any human or animal material including, but not limited to, excreta, secreta, blood and its components, tissue, and tissue fluids being shipped for purposes of diagnosis.

(3) A "biological product" means a biological product prepared and manufactured in accordance with the provisions of 9 CFR Part 10, Licensed Veterinary Biological Products, 42 CFR Part 73, Licensed Human Biological Products, 21 CFR 130.3, *New drugs for investigational use in humans*, 9 CFR Part 103, Biological Products for Experimental Treatment of Animals, or 21 CFR 130.3(a), *New drugs for investigational use in animals*, and which, in accordance with such provisions, may be shipped in interstate traffic.

(b) *Transportation; etiologic agent minimum packaging requirements.* No person may knowingly transport or cause to be transported in interstate traffic, directly or indirectly, any material, including but not limited to, diagnostic specimens and biological products, containing, or reasonably believed by such person to contain, an etiologic agent unless such material is packaged to withstand leakage of contents, shocks, pressure changes, and other conditions incident to ordinary handling in transportation.

(c) *Transportation; etiologic agents subject to additional requirements.* No person may knowingly transport or cause to be transported in interstate traffic, directly or indirectly, any material, other than diagnostic specimens and biological products, containing, or reasonably believed by such person to contain, one or more of the following etiologic agents unless such material is packaged in accordance with the requirements specified in paragraph (b) of this section, and unless, in addition, such material is packaged and shipped in accordance with the requirements specified in subparagraphs (1)–(6) of this paragraph:

BACTERIAL AGENTS

*Actinobacillus*—all species.
*Arizona hinshawii*—all serotypes.
*Bacillus anthracis.*
*Bartonella*—all species.
*Bordetella*—all species.
*Borrelia recurrentis, B. vincenti.*
*Brucella*—all species.
*Clostridium botulinum, Cl. chauvoei, Cl. haemolyticum, Cl. histolyticum, Cl. novyi, Cl. septicum, Cl. tetani.*
*Corynebacterium diphtheriae C, equi, C. haemolyticum, C. pseudotuberculosis, C. pyogenes, C. renale.*
*Diplococcus (Streptococcus) pneumoniae.*
*Erysipelothrix insidiosa.*
*Escherichia coli,* all enteropathogenic serotypes.
*Francisella (Pasteurella) tularensis.*
*Haemophilus ducreyi, H. influenzae.*
*Herellea vaginicola.*
*Klebsiella*—all species and all serotypes.
*Leptospira interrogans*—all serotypes.
*Listeria*—all species.
*Mima polymorpha.*
*Moraxella*—all species.
*Mycobacterium*—all species.
*Mycoplasma*—all species.
*Neisseria gonorrhoeae, N. meningitidis.*
*Pasteurella*—all species.
*Pseudomonas pseudomallei.*
*Salmonella*—all species and all serotypes.
*Shigella*—all species and all serotypes.
*Sphaerophorus necrophorus.*
*Staphylococcus aureus.*
*Streptobacillus moniliformis.*
*Streptococcus pyogenes.*
*Treponema careteum, T. pallidum,* and *T. pertenue.*
*Vibrio fetus, V. comma,* including biotype El Tor, and *V. parahemolyticus.*
*Yersenia (Pasteurella) pestis.*

FUNGAL AGENTS

*Actinomycetes* (including *Nocardia* species, *Actinomyces* species and *Arachnia propionica*).
*Blastomyces dermatitidis.*
*Coccidioides immitis.*
*Cryptococcus neoformans.*
*Histoplasma capsulatum.*
*Paracoccidioides brasiliensis.*

VIRAL, RICKETTSIAL, AND CHLAMYDIAL AGENTS

*Adenoviruses*—human—all types.
*Arboviruses.*
*Coxiella burnetii.*
*Coxsackie A* and *B viruses*—all types.
*Cytomegaloviruses.*
*Dengue virus.*
*Echoviruses*—all types.
*Encephalomyocarditis virus.*
*Hemorrhagic fever agents,* including *Crimean hemorrhagic fever (Congo), Junin,* and *Machupo viruses,* and others as yet undefined.
*Hepatitis-associated antigen.*
*Herpesvirus*—all members.
*Infectious bronchitis-like virus.*
*Influenza viruses*—all types.
*Lassa virus.*
*Lymphocytic choriomeningitis virus.*
*Marburg virus.*
*Measles virus.*
*Mumps virus.*
*Parainfluenza viruses*—all types.
*Polioviruses*—all types.
*Poxviruses*—all members.
*Psittacosis - Ornithosis - Trachoma-Lymphogranuloma* group of agents.
*Rabies virus*—all strains.
*Reoviruses*—all types.
*Respiratory syncytial virus.*
*Rhinoviruses*—all types.
*Rickettsia*—all species.
*Rubella virus.*
*Simian viruses*—all types.
*Tick-borne encephalitis virus* complex, including *Russian spring-summer encephalitis, Kyasanur forest disease, Omsk hemorrhagic fever,* and *Central European encephalitis viruses.*
*Vaccinia virus.*
*Varicella virus.*
*Variola major* and *Variola minor viruses.*
*Vesicular stomatis virus.*
*Yellow fever virus.*

(1) *Volume less than 50 ml.* Material shall be placed in a securely closed, watertight container (primary container (test tube, vial, etc.)) which shall be enclosed in a second, durable watertight container (secondary container). Several primary containers may be enclosed in a single secondary container, if the total volume of all the primary containers so enclosed does not exceed 50 ml. The space at the top, bottom, and sides between the primary and secondary containers shall contain sufficient nonparticulate absorbent material to absorb the entire contents of the primary container(s) in case of breakage or leakage. Each set of primary and secondary containers shall then be enclosed in an outer shipping container constructed of corrugated fiberboard, cardboard, wood, or other material of equivalent strength.

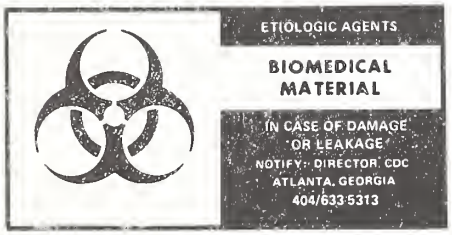
(2) *Volume 50 ml. or greater.* Packaging of material in volumes of 50 ml. or more shall include, in addition, a shock absorbent material, in volume at least equal to that of the absorbent material

[1] The requirements of this section are in addition to and not in lieu of any other packaging or other requirements for the transportation of etiologic agents in interstate traffic prescribed by the Department of Transportation and other agencies of the Federal Government.

between the primary and secondary containers, at the top, bottom, and sides between the secondary container and the outer shipping container. Single primary containers shall not contain more than 500 ml. of material. However, two or more primary containers whose combined volumes do not exceed 500 ml. may be placed in a single, secondary container. Not more than eight secondary shipping containers may be enclosed in a single outer shipping container. (The maximum amount of etiologic agent which may be enclosed within a single outer shipping container shall not exceed 4,000 ml.)

(3) *Dry ice.* If dry ice is used as a refrigerant, it must be placed outside the secondary container(s). If dry ice is used between the secondary container and the outer shipping container, the shock absorbent material shall be so placed that the secondary container does not become loose inside the outer shipping container as the dry ice sublimates.

(4) *Labels.* The label for Etiologic Agents/Biomedical Material, except for size and color, must be as shown:




(i) The color of material on which the label is printed must be white and the symbol and printing in red.

(ii) The label must be a rectangle measuring 51 mm. (2 inches) high by 102.5 mm. (4 inches) long.

(iii) The red symbol measuring 38 mm. (1½ inches) in diameter must be centered in a white square measuring 51 mm. (2 inches) on each side.

(iv) Type size of the letters of label shall be as follows:

| | |
|---|---|
| ETIOLOGIC AGENT | 10 pt. rev. |
| BIOMEDICAL MATERIAL | 14 pt. |
| IN CASE OF DAMAGE OR LEAKAGE | 10 pt. rev. |
| NOTIFY DIRECTOR CDC ATLANTA, GA | 8 pt. rev. |
| 404 633 5313 | 10 pt. rev. |

(5) *Damaged packages.* Carriers shall promptly, upon discovery of damage to the package that indicates damage to the primary container, isolate the package and notify the Director, Center for Disease Control, 1600 Clifton Road NE., Atlanta, GA 30333 (telephone (404) 633–5313), and the sender.

(6) *Registered mail or equivalent system.* Transportation of the following etiologic agents shall be by registered mail or an equivalent system which requires or provides for sending notification to the shipper immediately upon delivery:

*Actinobacillus mallei.*
*Coccidioides immitis.*
*Francisella (Pasteurella) tularensis.*
*Hemorrhagic fever agents,* including, but not limited to, *Crimean hemorrhagic fever (Congo), Junin, Machupo viruses.*
*Herpesvirus simiae* (B virus).
*Histoplasma capsulatum.*
*Lassa virus.*
*Marburg virus.*
*Pseudomonas pseudomallei.*
*Tick-borne encephalitis virus* complex, including, but not limited to, *Russian spring-summer encephalitis, Kyasanur forest disease, Omsk hemorrhagic fever,* and *Central European encephalitis viruses, Variola minor* and *Variola major.*
*Yersenia (Pasteurella) pestis.*

(d) *Notice of delivery; failure to receive.* When notice of delivery of agents containing, or suspected of containing, etiologic agents listed in paragraph (c)(6) of this section is not received by the sender within 5 days following anticipated delivery of the package, the shipper shall notify the Director, Center for Disease Control, 1600 Clifton Road NE., Atlanta, GA 30333 (telephone (404) 633–5313).

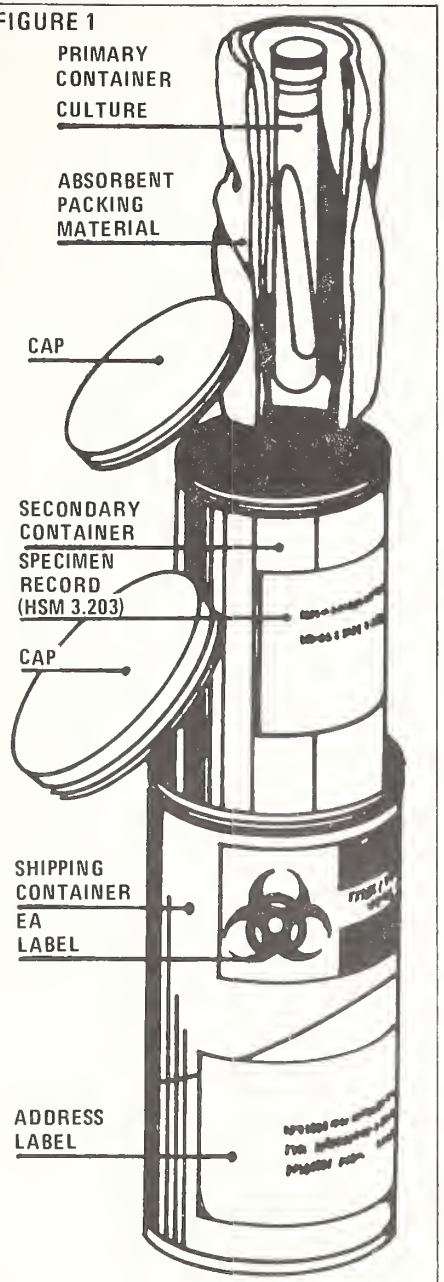
(e) *Requirements; variations.* The Administrator may approve variations from the requirements of this section if, upon review and evaluation, he finds that such variations provide protection at least equivalent to that provided by compliance with the requirements specified in this section and makes such findings a matter of official record.

(Sec. 361, 58 Stat. 703; 42 U.S.C. 264)

[FR Doc.72-9887 Filed 6-29-72;8:45 am]

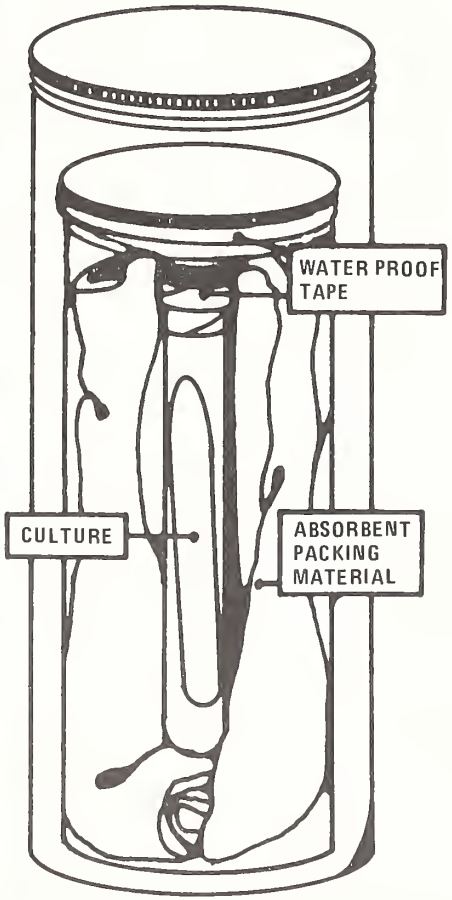
Effective July 30, 1972

FIGURE 1




# PACKAGING AND LABELING OF ETIOLOGIC AGENTS

FIGURE 2




CROSS SECTION
OF PROPER PACKING

The Interstate Quarantine Regulations (42 CFR, Part 72.25 Etiologic Agents) was revised July 31, 1972 to provide for packaging and labeling requirements for etiologic agents and certain other materials shipped in interstate traffic.

Figures 1 and 2 diagram the packaging and labeling of etiologic agents in volumes of less than 50 ml. in accordance with the provisions of subparagraph (C) (1) of the cited regulation. Figure 3 illustrates the color and size of the label, described in subparagraph (C) (4) of the regulations, which shall be affixed to all shipments of etiologic agents.

For further information on any provision of this regulation contact:

Center for Disease Control
Attn: Biohazards Control Office
1600 Clifton Road
Atlanta, Georgia 30333

Telephone: 404 – 633-3311

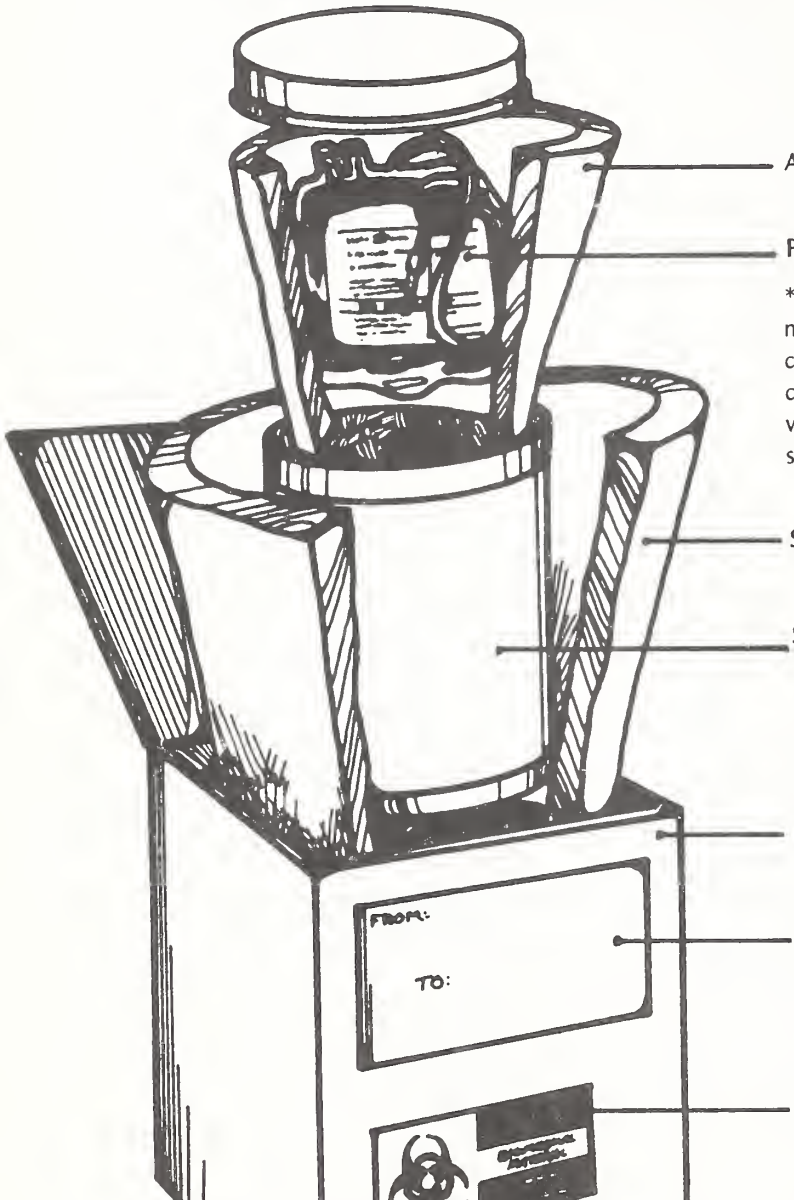
FIGURE 3

ETIOLOGIC AGENTS

**BIOMEDICAL MATERIAL**

IN CASE OF DAMAGE OR LEAKAGE
NOTIFY DIRECTOR, CDC
ATLANTA, GEORGIA
404/633-5313

# PACKAGING AND LABELING OF ETIOLOGIC AGENTS




ABSORBENT PACKING MATERIAL

PRIMARY CONTAINER *(Bottle, blood bag, etc.)**

*NOTE: Single primary containers may not exceed 500 ml. of material. Two or more primary containers whose combined volumes do not exceed 500 ml. may be enclosed in a single, secondary container. The maximum volume of etiologic agent which may be enclosed in a single outer shipping container shall not exceed 4000 ml.

SHOCK ABSORBENT MATERIAL

SECONDARY CONTAINER *(Gasketed screwcap with waterproof tape or hermetically sealed can)*

OUTER SHIPPING CONTAINER

MAILING LABEL

ETIOLOGIC AGENT LABEL

The Interstate Quarantine Regulations (42 CFR, Part 72.25, Etiologic Agents) was revised July 31, 1972, to provide for packaging and labeling requirements for etiologic agents and certain other materials shipped in interstate traffic. The illustration shows acceptable packaging and labeling of etiologic agents in accordance with subparagraphs (c) (2) and (4) of the cited regulation.

For further information on any provision of this regulation contact:

Center for Disease Control
Attn: Biohazards Control Office
1600 Clifton Road
Atlanta, Georgia 30333

Telephone: 404-633-3311

## XI. Training Aids, Materials and Courses

### A. Slide-Tape Cassettes

1. Assessment of Risk in the Cancer Virus Laboratory ($10).
2. Effective Use of The Laminar Flow Biological Safety Cabinet ($10).
3. Formaldehyde Decontamination of Laminar Flow Biological Safety Cabinets ($10).
4. Certification of Class II (Laminar Flow) Biological Safety Cabinets ($13).
5. Hazard Control in the Animal Laboratory ($10).
6. Basic Principles of Contamination Control (In preparation).
7. Selection of a Biological Safety Cabinet (In preparation).

These slide tape cassettes are available for purchase from the National Audiovisual Center. The price for each is given above after the title. Send your order prepaid with a check or money order made payable to National Archives Trust Fund and mail to: Sales Branch, National Audiovisual Center (GSA), Washington, D.C. 20409.

8. Research Laboratory Safety

This slide tape cassette, stock number 176.79, is available for $75 from the National Safety Council, 425 North Michigan Avenue, Chicago, Illinois 60611.

### B. Films

1. Air Sampling for Microbiological Particulates (M-926).
2. Handling the Laboratory Guinea Pig (T2618-X).
3. Handling the Laboratory Mouse (T2617-X).

4. Infectious Hazards of Bacteriological Techniques (M-382).
5. Laboratory Design for Microbiological Safety (M-1091).
6. Plastic Isolators: New Tools for Medical Research (M-599).
7. Safe Handling of Laboratory Animals (M-455).
8. Surface Sampling for Microorganisms (Rodac Method) (M-924).
9. Surface Sampling for Microorganisms (Swab Method) (M-925).

These films are available on loan without charge from:

Media Resources Branch
National Medical Audiovisual Center (Annex)
Station K
Atlanta, Georgia 30324

The same films (except 2 and 3) can be rented or bought from:

National Audiovisual Center (GSA)
(Rental Branch) - (Sales Branch)
Washington, D.C. 20409

C. Courses

1. Biohazard and Injury Control in the Biomedical Laboratory. Presented by the University of Minnesota, School of Public Health and the National Cancer Institute, Office of Research Safety. Direct inquiries to Dr. Donald Vesley, University of Minnesota, School of Public Health, 1325 Mayo Memorial Building, Minneapolis, Minnesota 55455.

| | |
|---|---|
| June 22-24, 1976 | Los Angeles, CA |
| October 26-28, 1976 | Boston, MA |
| December 7-9, 1976 | Bethesda, MD |

2. Biohazard Containment and Control for Recombinant DNA Molecules.

   Presented by the University of Minnesota, School of Public Health and the National Cancer Institute, Office of Research Safety. Direct inquires as above.

   September 8-9, 1976 Stanford, CA

   September 21-11, 1976 Cold Spring Harbor, NY

3. Safety in Laboratory.

   Presented by National Institute of Occupational Safety and Health, Division of Training and Manpower Development, by special arrangement. Robert A. Taft Laboratories, 4676 Columbia Parkway, Cincinnati, Ohio 45226.

4. Laboratory Safety Management.

   Presented by the Laboratory and Training Division, Bureau of Laboratories, Center for Disease Control, Atlanta, Georgia

   September 14-16, 1976

   September 13-15, 1977

XII. Outline of a Safety and Operation Manual for a P4 Facility

A. Purpose

B. Policy

C. Responsibility and Authority

1. Management

2. Supervisor

3. Each Employee

4. Facility Safety Officer

5. Biohazard Safety Committee

D. Facility Assignment Procedures

E. Reporting of Major and Minor Accidents and Injuries, Exposure to Toxic or Infectious Materials, Unsafe Conditions and Property Damages, and Rendering First-Aid.

F. General Laboratory Safety

1. Fire

2. Equipment

3. Physical

4. Chemical

5. Radiological

G. Safety Procedures Associated with Biohazard Activities of the Laboratory

1. Personnel Practices

2. Operational Practices

H. Medical Surveillance

I. Facility Operations

1. Personnel Access Procedures
2. Access Procedures for Equipment Materials and Supplies
3. Maintenance and Support
4. Zone Classification
5. Facility Monitoring Procedures
6. Housekeeping

J. Others

1. Packaging and Shipment of Biohazardous Materials
2. Emergency Procedures
3. Insect and Rodent Control
4. Orientation and Training

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Appendix D was prepared by a Working Group Consisting of:

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James F. Sullivan
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Laboratories

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